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I. The Anatomy and Function of the So-Called 'Notched Organs' of Nuttall and Shipley on the Thorax of Larvae of *Anopheles quadrimaculatus*

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(Received for publication 14 May 1951)

In a study of the force or forces involved in the floating of anopheline larvae at the water surface by the use of a metallographic microscope, our attention was drawn to the presence on photomicrographs of a pair of remarkable ear-like structures, one situated antero-laterally on each side of the dorsum of the thorax of larvae of the first to the fourth instar. It was also found that although these two structures were, in a large part, so membranous and transparent that they could easily be overlooked under a direct-light-illuminated microscope, they played an important part in the floating mechanism of these larvae. A detailed study was therefore made, and it was found that these structures are really the future respiratory trumpets of the pupa. In view of the anatomical and functional importance of these structures, it is felt desirable to report our findings in order to bring them into prominence in American literature and to clear up doubts about these structures as to their anatomy and function.

A review of the literature revealed that as early as 1901, Nuttall and Shipley reported the existence of a pair of 'notched organs' on the thorax of anopheline larvae. These authors elaborated somewhat their findings in the subsequent two years. In 1921 Iyengar reported his findings in a closer study of these structures and stated later (1928) that their function was to prevent the thorax of the larvae from following the rotational movement of the head. MacGregor (1927) noted that he had seen these structures in anopheline, but not in culicine, larvae, and thought they served as attachments to the surface film to support the thorax in the horizontal position assumed by the anopheline larvae. MacGregor also mentioned that Alcock had encountered these structures in culicine larvae. Marshall (1938) thought that these structures acted to support the weight of the larvae when they were at the surface of the water.

MATERIALS AND PROCEDURES

The eggs of *Anopheles quadrimaculatus* were obtained through the kindness of Dr. W. V. King, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Orlando, Florida. The larvae hatched from these eggs were used in the present as well as in the subsequent studies. The larvae were viewed through a standard Bausch and Lomb metallographic microscope so that they could be illuminated simultaneously from above and below. The metallographic microscope is so constructed that the illumination is contained in the optical system of the microscope and the specimen is illuminated from above. Thus the image is produced in the eyepiece by reflected light and, in the case of a solid object like an anopheline larva

floating at the surface of a liquid, it is easy to distinguish the boundaries of that part of the larva which is above the water line.

By using a tank with a transparent bottom it was possible to illuminate the larvae from beneath and in this manner to produce a better shadow image. To facilitate photomicrography, it was necessary to immobilize the larva for a given period of time. To accomplish this, each larva was placed in ice-water for a few minutes before it was introduced into the tank. The larvae were not injured but were effectively immobilized by cold. Pictures were taken on high contrast films with a camera attached to the microscope. Aerosol-OT, an anionic detergent, was used at different concentrations to maintain the larvae at different levels of their floating position.

To study the anatomy of these ear-like structures on the thorax, the larvae were examined under a direct-light-illuminated microscope. In order to bring out the membranous part of these structures, the suspending water was tinged with a dye like malachite green. To trace the relation of the ear-like structures to the respiratory system, kerosene colored with Oil Red O (Sudan II) was used to bring the tracheal tubes into vision.

RESULTS AND DISCUSSION

As shown in figures 1, 2, and 2a, this pair of ear-like organs was present on the thorax of larvae of all developmental stages, and was able to emerge from the water even when the surface tension was 39.5 dynes per cm. Also, as shown in figures 3 and 4, each of these organs consists of a dark-colored 'cup' located inside the thorax and a notched, transparent, membranous part around the edge of the cup and protruded out of the thorax. The membranous part of these organs is so highly refractile that it is easily overlooked in direct-light-illuminated microscopy. It is due to apparently the water-repellent property of this membranous part that enables it to emerge out of the water surface.

In the pictures of the second and fourth instar shown in figures 2 and 2a, the shadow images of these organs are much more conspicuous than the images viewed in a direct-light-illuminated microscope. The fluffy appearance, suggesting a brush-like structure, is rather misleading, and was apparently due to the difference in the degree of refraction of the membranous part. It should be noted that because of the higher surface tension of the suspending fluids used for floating the larvae pictured in figures 2 and 2a, the membranous part of these organs protruded farther above the water surface and, therefore, produced a clearer shadow picture than it did in the smaller larva pictured in figure 1.

The anatomical relationship of these two organs to the respiratory system of the larva is shown in figures 3 and 4. These were obtained from a series of examinations of larvae suspended in colored-water, as well as of larvae that were treated with Sudan II dyed-kerosene to demonstrate the tracheae. It is apparent that these two notched organs are nothing but the future respiratory trumpets of the pupa. Furthermore, repeated examinations of larvae that were about to molt, as well as those that were molting, revealed the disappearance of the membranous part and the depressing of the antero-lateral corner on each dorsal side of the thorax, thus bringing the cup

part of these organs out of the thorax, to become the respiratory trumpets of the pupa. Hence, it is felt not inappropriate to call these two ear-like organs the prepupal respiratory trumpets.

FIG. 1. 1st instar larva of *A. quadrimaculatus* floating on water surface (σ = 39.5 dynes/cm). × 60.

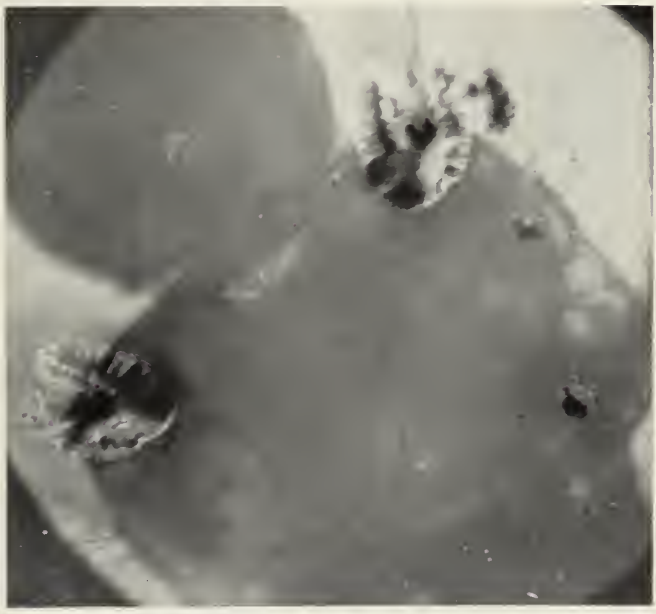

FIG. 2. 2nd instar larva of *A. quadrimaculatus* floating on water surface (σ = 63 dynes/cm). × 60

It should be noted that these prepupal trumpets are shut off from the tracheae by the closed ends of a tubular section that joins the bottom of the cup to the main

trachea on each side of the thorax (see figure 4). This anatomical relationship is brought out by the fact that in larvae treated with colored-kerosene, the tubular

FIG. 2a. 4th instar larva of *A. quadrimaculatus* floating on water surface (σ = 56 dynes/cm). × 60.

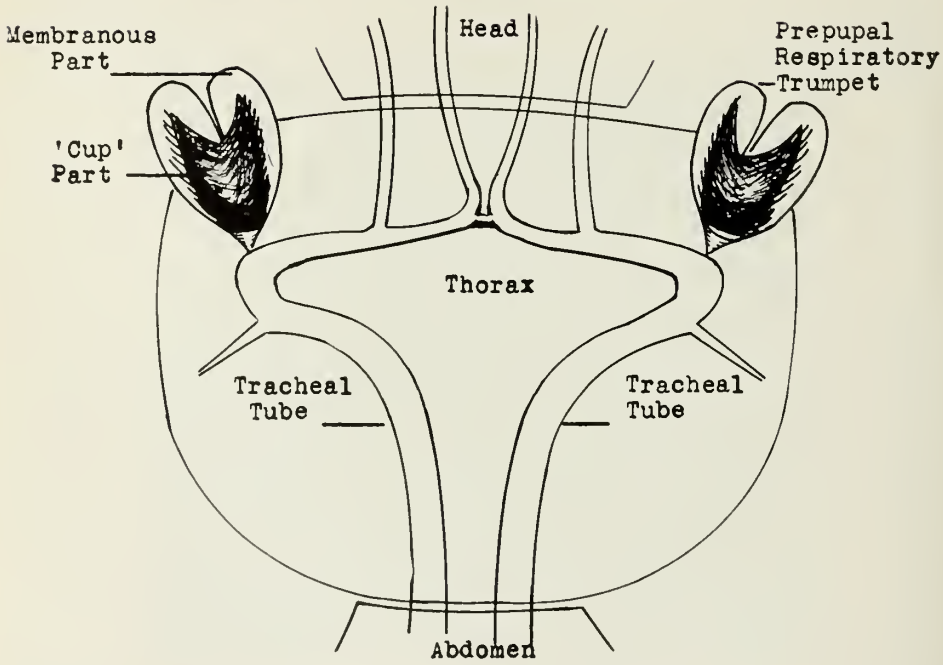

FIG. 3. Dorsal view of Thorax of *A. quadrimaculatus* Larva

section is unstained, while the tracheae and their branches are stained red, and the cups are filled to their bottom with the colored-kerosene. It is also of interest to note that as soon as these cups are filled with oil, they start to contract, and expel their contents. From both the dorsal and the lateral view of the prepupal trumpets, it

seems apparent that their membranous part is not in the same plane with the walls of the cup, and is curved outward to form a rounded edge.

In larvae of *Aedes aegypti* (hatched from eggs also obtained from Dr. W. V. King), similar membranous part of the prepupal trumpets was not seen, but the cup part is present in antero-lateral part of each side of the thorax. It seems apparent that they are also the future trumpets of the pupa. The lack of a membranous part protruding from the thorax in the *Aedes* larvae can be readily explained by the fact that these larvae, being bottom-feeders, need no organs to keep the anterior part of the body floating at the water surface. Hence, it seems that what Alcock (according to MacGregor) observed in culicine larvae may have been these cup-like structures.

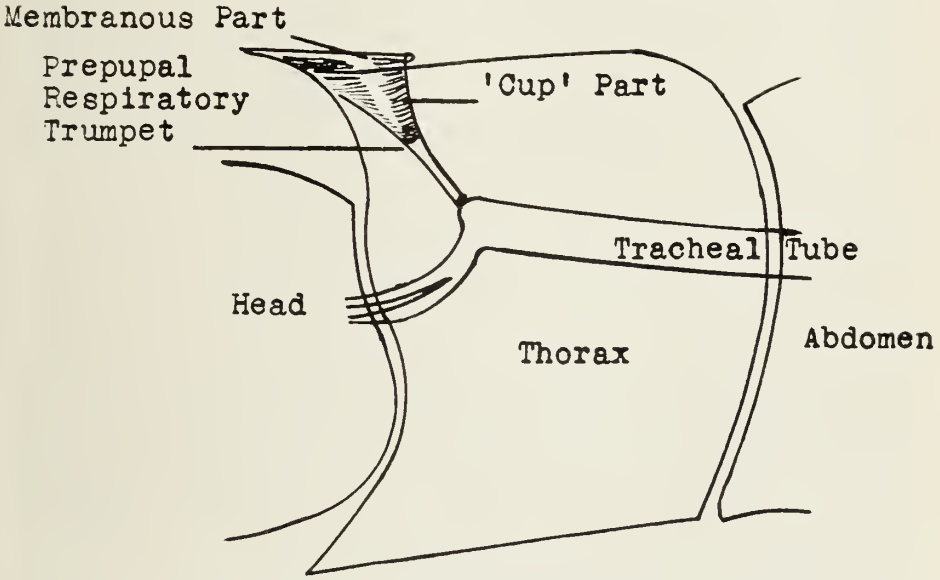

FIG. 4. Lateral view of Thorax of *A. quadrimaculatus* Larva

SUMMARY AND CONCLUSION

During a study of the mechanism involved in floating of anopheline larvae at a water surface, a pair of ear-like structures, located antero-laterally on each side of the dorsum of the thorax, was found in all four instars of *A. quadrimaculatus* larvae. The part protruding out of the thorax is membranous and transparent, and is apparently the only structure which enables the larva to float the anterior part of its body at water surface. It is therefore very important in the feeding mechanism of the anopheline larvae.

A detailed study of the anatomical relationship of these structures to the tracheal tubes in both the larvae and the larvo-pupal forms revealed that they are connected to the two main tracheae at the 'bend' in the thorax by a tubular section with closed ends, which becomes clear toward the end of the larvo-pupal stage. Hence, it seems apparent that this pair of ear-like structures are the prepupal respiratory trumpets.

Although the membranous part of the two prepupal trumpets that protrudes from the thorax in anopheline larvae was not found in *Aedes aegypti* larvae, the cup part

of these structures was seen in the antero-lateral part of each side of the thorax, and seemed to have the same anatomical relations to the tracheal tubes as in anopheline larvae.

It is concluded that these two ear-like structures on the thorax of anopheline larvae are not only the prepupal respiratory trumpets, but are also organs enabling the larvae to float the anterior part of their body. Furthermore, it is concluded that a pair of prepupal trumpets exists in *Aedes* (and probably in *Culex*) larvae, but in these latter the membranous part that protrudes out of the thorax is lacking.

REFERENCES

Nuttall, G. H. F., and Shipley, A. E. 1901. Studies in relation to Malaria. II. The structure and biology of Anopheles (*Anopheles maculipennis*). J. Hyg., **1:** 45–77, 269–276, 451–484.

Nuttall, G. H. F., and Shipley, A. E. 1902. Studies in relation to Malaria. II. The structure and biology of Anopheles (*Anopheles maculipennis*). *Ibid.*, **2:** 58–84.

Nuttall, G. H. F., and Shipley, A. E. 1903. Studies in relation to Malaria. II. The structure and biology of Anopheles (*Anopheles maculipennis* Meigen). *Ibid.*, **3:** 166–215.

Iyengar, M. O. T. 1921. A preliminary note on new thoracic appendages in anopheline larvae. Rept. Proc. 4th Entomol. Meeting, Pusa, Calcutta, India, 216–218.

Iyengar, M. O. T. 1928. Structure and function of the contractile thoracic appendages of the *Anopheles* larvae. Indian J. M. Research, **16:** 281–296.

MacGregor, M. E. 1927. Mosquito Surveys. Bailliere, Tindall and Cox, London, 293 pp.

Marshall, J. F. 1938. The British Mosquitoes. British Museum, London, 341 pp.

RESUMEN Y CONCLUSION

Durante un estudio del mecanismo que permite la flotación de larvas anofelinas en la superficie del agua encontramos en las cuatro fases larvarias de *A. quadrimaculatus* un par de estructuras pareciads a orejas situadas a cada lado del dorso del tórax en la porción antero-lateral. La parte que proyecta del tórax es membranosa y transparente y aparentemente es la única estructura que permite que la larva flote la parte anterior de su cuerpo en la superficie del agua. Es por lo tanto muy importante en el mecanismo de alimentación de las larvas anofelinas.

Un estudio detallado de la relación anatómica de estas estructuras y los conductos traqueales en las formas larvales y las larvopupales reveló que éstas están conectadas a las dos traqueas principales en la región encorvada del tórax por medio de una estructura tubular de extremos cerrados que se manifiesta claramente a fines de la etapa evolutiva larvo-pupal. Aparentemente este par de estructuras que parecen orejas son las "trompetas" respiratorias prepupales.

Aunque la parte membranosa de cada una de las dos "trompetas" prepupales que proyectan del tórax en larvas anofelinas no fueron encontradas en larvas de *Aedes aegypti*, la parte acopada de estas estructuras se observó en la parte antero-lateral de cada lado del tórax, y pareció tener las mismas relaciones antatómicas con los conductos traqueales que las larvas anofelinas.

Se concluye que estas dos estructuras que parecen orejas en el tórax de larvas anofelinas no solamente son las "trompetas" respiratorias prepupales pero también organos que permiten que las larvas puedan flotar la parte anterior de sus cuerpos. Se concluye además que existe en las larvas *Aedes* (y probablemente en *Culex*) un par de "trompetas" prepupales pero éstas carecen de la parte membranosa que proyecta del tórax.

STUDIES ON ANOPHELINE LARVAE

II. The Mechanism Involved in the Flotation of Larvae of *A. quadrimaculatus* on a Water Surface

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It is generally believed that the flotation of anopheline larvae at a water surface is facilitated by their palmate hairs. The presence of these hairs, however, does not explain how the larva floats the anterior part of its body, since they are found only on the dorsum of the abdomen and may be absent on the first, and sometimes on the second abdominal segments. Furthermore, the small size of each tuft of these hairs compared with the whole body (see Figure 10 and 11) and the shape of each hair make it doubtful that these hairs alone can support the larvae during breathing and feeding at the water surface.

It occurred to us that the respiratory apparatus on the dorsum of the eighth abdominal segment, and possibly other structures, might be involved in the floating of these larvae. Hence, a study was made of the force or forces involved in the flotation of *Anopheles quadrimaculatus* larvae. In this study it was revealed that both the prepupal respiratory trumpets on the thorax and the posterior respiratory apparatus on the eighth abdominal segment play a more important role than the palmate hairs in the floating of anopheline larvae. The present report presents the data and the analysis of the data. For materials and procedures used, the reader is referred to the preceding paper.

Mathematical Consideration of the Forces Involved in the Floating of Anopheline Larvae at a Water Surface

The fact that anopheline larvae have a specific gravity greater than that of water and that they have nothing like the air-sac of the fish for buoyancy make it apparent that they will sink in water unless the gravitational force is counteracted by an upward-pulling force. When immersed, these larvae depend on the 'kicking' movement of the body to swim sideward or upward. Once the larva manages to get to the water surface, it can remain there indefinitely. Hence, it must be the surface energy force that the larva is utilizing for flotation. A mathematical consideration of the forces existing at the solid-liquid, solid-air, and air-liquid interfaces is therefore given under the following two headings:

1. The 'Wetting' of a Solid by a Liquid. When a solid is brought into contact with a liquid, the solid will be 'wetted' if the intermolecular attraction between the molecules in the liquid and those in the solid at the interface is greater than that between the molecules in the liquid itself. For practical purposes, this wetting phenomenon may be considered to be a manifestation of the forces or tensions at the liquid-air, liquid-solid, and solid-air interfaces, as illustrated in figure 1.

The arrows F_1, F_2, and F_3 in figure 1 indicate the directions of the forces operating

at these interfaces. These three forces intersect at point C. At equilibrium, the resultant force at C is zero, or

$$F_2 = F_1 + F_3 \cdot \cos \theta \tag{1}$$

Converting these forces into interfacial tensions, it becomes

$$\gamma_S = \gamma_{LS} + \gamma_L \cdot \cos \theta \tag{2}$$

or

$$\cos \theta = \frac{\gamma_S - \gamma_{LS}}{\gamma_L} \tag{3}$$

or

$$\theta = \frac{\gamma_S - \gamma_{LS}}{\gamma_L} \cdot \cos^{-1} \tag{4}$$

where γ_S is the surface tension of the solid; γ_L is the surface tension of the liquid; γ_{LS} is the solid-liquid interfacial tension; and θ is the angle of contact.

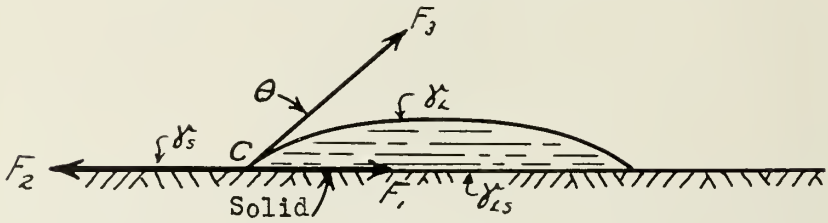

FIG. 1. Diagram of forces acting at different interfaces of a solid in contact with a liquid.

From equation (4) it becomes apparent that the angle of contact, θ, is a function of all three forces operating at these interfaces, and that measurements of this angle take into consideration all these forces, and determine whether or not the liquid will wet the solid. For instance, if the angle of contact is 0°, then the surface tensions of the solid is equal to the sum of the surface tension of the liquid and the interfacial tension of the solid-liquid interface; the molecules in the liquid attract the molecules in the solid just as much as they attract themselves. The angle of contact will also be 0° if the molecules in liquid attract those in the solid more than they attract themselves.

An angle of contact of 90° therefore indicates that the molecules in the liquid attract those in the solid only half as much as they attract themselves; an angle of 180° indicates that there is no attraction between the molecules in the liquid and those in the solid.

2. Floating of a Solid by Interfacial Tension at a Liquid Surface. When a solid of high specific gravity is placed on the surface of a liquid of low specific gravity, the solid will float on the liquid surface if the angle of contact, θ, is greater than 0°, that is, if the solid is not wetted, and if that portion of the weight of the solid that is unbalanced by the weight of the displaced water can be supported by the surface tension of the liquid. For instance, if a paraffin-coated needle is placed on a water surface, the needle will float, and the water surface under the needle will be depressed

to a point where the vertical component of the surface tension becomes great enough to support the unbalanced weight of the needle. At equilibrium, the condition, as illustrated in figure 2, can be expressed by the equation

$$W_n - W_s = \gamma_w \cdot p \cdot \sin \alpha \qquad (5)$$

where W_n = the weight of the needle in air
W_s = the weight of the displaced water
γ_w = the surface tension of water
p = the perimeter of the needle at the waterline
α = the angle of depression, the angle between the horizontal water surface and the water surface at the point of contact

In figure 2, the needle is shown floating with the waterline a little below its horizontal diameter. However, this condition does not actually exist, since the angle of

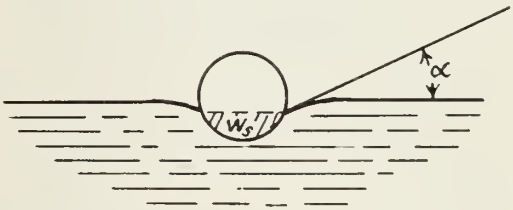

FIG. 2. The floating of a solid on a liquid

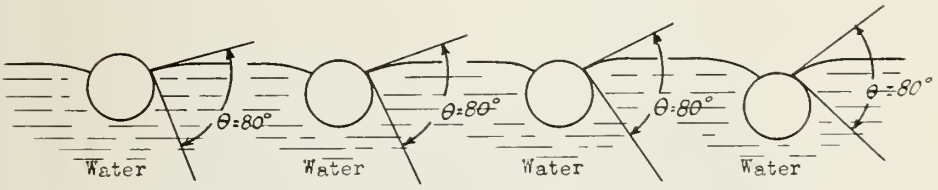

FIG. 3. Positions of a needle floating in a liquid when the angle of contact of liquid to solid (θ) is 80°.

contact between paraffin and water is 106° (the largest contact angle between a smooth solid and water) and the needle would float at a level low enough to have the maximum angle of contact obtainable for these two surfaces. Assuming that the actual angle of contact is 80°, then the needle may float in several positions as shown by the four diagrams in figure 3. The heavier the needle, the lower will it float. Hence, it becomes clear that the level at which a solid floats at a liquid surface is determined by (a) the angle of contact, θ, and (b) the angle of depression, α. It also becomes clear that there must be a compatible relationship between the angles, θ and α.

For a given solid floating on a liquid, a certain angle of contact, θ, will exist, regardless of the vertical oscillations of the solid at the liquid surface (disregarding the 'hysteresis' effect on θ). Also, for a given floating solid, some shape of cross-section will exist and, at any point of this cross-section, a slope $\frac{dy}{dx}$ will exist with reference to the horizontal plane as shown in figure 4.

From the diagram shown in figure 4 it is clear that the relationship of the angle of depression, α, to the angle of contact, θ, can be expressed by the equation

$$\alpha = \tan^{-1}\left(\frac{dy}{dx}\right) + \theta \qquad (6)$$

If the weight of the solid is increased, or the angle of contact is decreased, the solid will sink in the liquid until a new position of equilibrium is established. When the angle θ reaches 0°, it is impossible to generate any lifting force by surface tension, since it is impossible to develop a positive value of α, and the solid will sink.

If the surface tension of the liquid is lowered, i.e. by the use of a detergent, the wetting property of the liquid will be increased and the lifting force decreased; then the angle of contact, θ, will become smaller and the angle of depression, α must be larger than for the original liquid, in order to float the solid. Hence, there must be a critical value of the surface tension of liquid, at which the angle of contact,

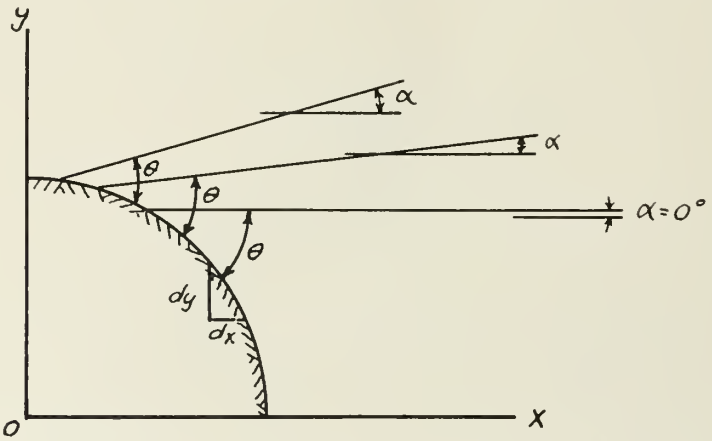

FIG. 4. Relationship of α to θ as a function of the shape of a floating solid

θ, will be 0°, and below which the solid will sink, as there will be no positive value for the angle of depression, α.

Experimental Data on Flotation of A. quadrimaculatus *Larvae on Water Surface with Varying Surface Tensions*

On the basis of the theoretical considerations given above, experiments were carried out with *A. quadrimaculatus* larvae in distilled water at 25°C dosed with varying amounts of Aerosol-OT (dioctyl sodium sulfosuccinate). Preliminary observations showed that the larvae (first to fourth instar) depend on the non-wetting property of three different anatomical structures for their ability to float at the water surface, namely: the posterior respiratory apparatus, the prepupal trumpets, and the palmate hairs. A surface tension of about 30 dynes per cm. (determined by a Cenco-Du Nouy Tensiometer) is the critical value for floating of the larvae. Below this value the larvae were unable to 'hang' themselves at the water surface. At surface tensions between 30 and 35 dynes per cm. the larvae hung themselves at the water surface by the posterior respiratory apparatus for a limited time, about 15

minutes. Oftentimes they failed to float in their first few attempts, and 'licked' the plates of the respiratory apparatus as if lubricating it to improve its non-wetting property. Not infrequently they succeeded in floating after these preliminary efforts; but invariably the respiratory apparatus gradually became flooded and the larvae sank.

At surface tensions between 39 and 49 dynes per cm. the larvae hung themselves at the water surface by both the posterior respiratory apparatus and the prepupal trumpets. The prepupal trumpets were apparently not needed to float the larvae during respiration, but were essential in floating for feeding purposes.

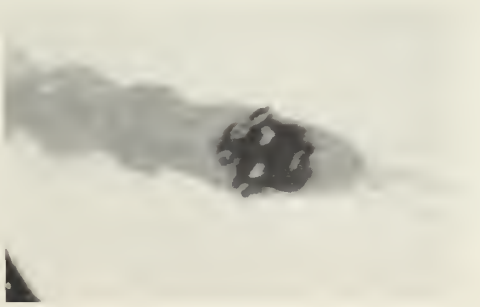

FIG. 5. Dorsum of posterior half of abdomen of first-instar larva floating on water surface (γ = 39.5 dynes/cm). × 60.

FIG. 6. Dorsum of posterior half of abdomen of second-instar larva floating on water surface (γ = 30.5 dynes/cm). × 60.

At surface tensions over 50 dynes per cm. the respiratory apparatus, the prepupal trumpets, and the palmate hairs all took part in supporting the larvae at the water surface.

In order to elaborate on these preliminary observations, and to facilitate a quantitative evaluation of the forces utilized by the larvae for their flotation, photomicrographs were taken through a metallographic microscope of the late first and second, and early fourth instar larvae floating at water surfaces with varying surface tensions. To compute the volume of each larva it was necessary to take two or more photomicrographs of different parts of each larva, to obtain a composite picture.

Figures 5 and 6 show the posterior part of a first and second instar larva, supported by the respiratory apparatus alone, at water surfaces with surface tensions of 39.5

and 30.5 dynes per cm., respectively. These pictures show clearly that the posterior respiratory apparatus of each larva was above the water surface. The plates forming the apparatus have an irregular contour, and hence the slope $\frac{dy}{dx}$ in equation (6) at various points around the perimeter of the apparatus is variable.

Figures 7 and 8 show the posterior part of a first and second instar larva, respectively, floating by the posterior respiratory apparatus, at a surface tension of 35

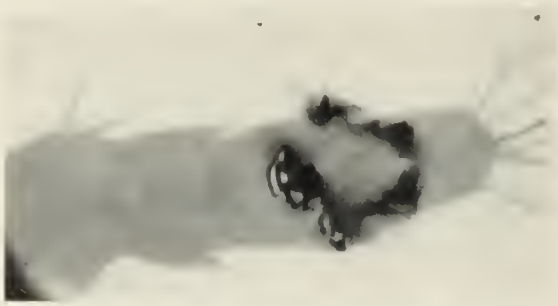

FIG. 7. Dorsum of posterior half of abdomen of second-instar larva floating on water surface (γ = 35 dynes/cm). × 60.

FIG. 8. Dorsum of posterior third of abdomen of second-instar larva floating on water surface (γ = 35 dynes/cm). × 60.

dynes per cm. Since these pictures were taken about 10 minutes after the larvae were placed in the solution, the central part of the apparatus of both larvae was flooded with water. The irregular contour of the apparatus is especially well illustrated. Since the tanks used in these experiments have a diameter of 5 cm., and were filled with water to a depth of only 3 to 4 mm., it seems quite possible that evaporation may have increased the concentration of Aerosol-OT, thus lowering the surface tension of the suspending fluid and causing the flooding of the apparatus.

As shown in figures 1, 2, and 3 of the preceding paper, which are photomicrographs of the anterior parts of the first, second, and fourth instar larvae floating at surfaces

tensions of 39.5, 63, and 56 dynes per cm. respectively, the prepupal trumpets were involved in the floating mechanism when the surface tensions were over 39 dynes per cm.

FIG. 9. Dorsum of abdomen of second-instar larva floating on water surface (γ = 57.5 dynes/cm). × 60.

FIG. 10. Dorsum of posterior two-thirds of abdomen of second-instar larva floating on water surface (γ = 57.5 dynes/cm). × 60.

In these three illustrations it is clearly seen that the larvae floated closer to the water surface with a surface tension of 56 or more dynes per cm. than with a tension of 39.5 dynes per cm., since the outline of the head and the thorax was much clearer at the higher than at the lower surface tensions. The shape of the prepupal trumpets in these pictures also show that they have more regular contour than the respiratory apparatus for the development of the angle of depression.

As illustrated in figures 9, 10, 11, and 12, the palmate hairs are involved in the

floating mechanism when the water surface tension is over 50 dynes per cm. It is of interest to point out that, as shown in figure 11, many of the tufts of palmate hairs may still remain under the water surface even at a surface tension of over 60 dynes per cm. The lanceolate shape of the hairs increases considerably the effective perimeter of each tuft but their small size, plus the fact that they seem to have less non-wetting properties than the prepupal trumpets, makes them much less effective for support in the flotation.

FIG. 11. Dorsum of abdomen of second-instar larva floating on water surface (γ = 60.5 dynes/cm). × 60

FIG. 12. Dorsum of posterior half of abdomen of second-instar larva floating on water surface (γ = 52.5 dynes/cm). × 60.

Computation of the Forces Involved in Flotation of Larvae of A. quadrimaculatus *on a Water Surface*

In order to compute the angle of depression, α, the specific gravity of the larvae and the volume and perimeter of the larvae of each developmental stage were determined.

To facilitate the use of Stokes' Law for determining the specific gravity of the larvae, it is necessary to obtain the different rates of sinking of the same larvae in the same position in liquids of known specific gravities and viscosities, since the

larve are far from being sperical in shape. Using the equation that expresses Stokes' Law

$$v_1 = \frac{1}{18} g \frac{P_s - P_1}{n_1} d^2 \tag{7}$$

and

$$v_2 = \frac{1}{18} g \frac{P_s - P_2}{n_2} d^2 \tag{8}$$

where v_1 and v_2 = the velocities of settling in cm. per second of the same particles in fluids 1 and 2
g = the acceleration due to gravity
P_s = the specific of the particles
P_1 and P_2 = the specific gravities of liquids 1 and 2
d = the diameter of particles, cm.
n_1 and $_2$ = the viscosities of liquids 1 and 2

and combining equations (7) and (8), we have

$$\frac{v_1}{v_2} = \frac{\frac{P_s - P_1}{1}}{\frac{P_s - P_2}{2}} \tag{9}$$

or

$$\frac{P_s - P_1}{P_s - P_2} = \frac{v_1}{v_2} \cdot \frac{n_1}{n_2} \tag{10}$$

Using distilled water and five and seven per cent glycerine at 25°C in glass tanks with dimensions of 10 × 10 × .8 cm., 20 determinations were made of the sinking rates of first-, second-, and fourth-instar larvae. All larvae were paralyzed by a short exposure to 50° to 55°C and the sinking rates of those that sank only in a vertical position were recorded. Knowing the specific gravities and viscosities of any two of these liquids, and the sinking rates of the same larvae in them, the specific gravity of the larvae was computed from equation 10. The specific gravity computed from these results is shown in table 1.

The data presented in table 1 show that the specific gravity of anopheline larvae is about the same in all developmental stages, about 1.031 at 25°C. This value is comparatively small for specific gravity of living organisms; its lowness may be explained by the presence of air in the tracheal tubes of the larvae. It is of some interest to note that larvae that had been previously 'drowned' in solutions of Aerosol-OT having surface tensions below 30 dynes per cm. sunk faster than those used in the specific gravity determinations.

The most convenient way of determining the volume of a larva is to divide its body into head, thorax, and abdomen, and to compute the volume of each part separately. Since the dorso-ventral and lateral diameters of the cross-section of the abdomen differ but slightly, the abdomen may be considered cylindrical in shape.

The thorax has a dorso-ventral diameter about the same as that of the first abdominal segment, but a much greater lateral diameter, and therefore is considered to be like an ellipsoidal cylinder. There was some difficulty in deciding on the shape of the head, to compute its volume. As its base is connected with the thorax in a rather straight line, and its anterior part is quite blunt (disregarding the mouth brushes), it seemed more accurate to consider the head as a cylinder rather than a sphere. The irregularity of the width of each part could be eliminated in computation by finding the area of each part and dividing it by the length. By this manipulation the average diameter of a cylinder may. be obtained.

The outline of a larva of each developmental stage was traced from the photomicrographs on to a piece of scaled tracing paper. The area of each part was ascertained and the average diameter computed. Employing the equation for computing the volume of a cylinder ($v = \pi\frac{d^2}{4}L$), and an ellipsoidal cylinder ($v = \frac{\pi d_1 d_2}{4} L$), the volume of each part of the larva was computed.

TABLE 1

Specific gravity of A. quadrimaculatus *larvae at 25°C*

STAGE OF *A. quadrimaculatus* LARVAE	SPECIFIC GRAVITY OF LARVAE DETERMINED IN	
	Distilled water and 5 per cent glycerine	5 per cent and 7 per cent glycerine
First instar	1.0330	1.0295
Second instar	1.0326	1.0307
Fourth instar	1.0305	1.0300

For computing the angle of depression, α, equation (5) was employed, which is

$$W_1 - W_w = \gamma_w \, p \sin \alpha$$

where W_1 = the weight of the larva in gm.
W_w = the weight of the liquid displaced in gm.
γ_w = the surface tension of the fluid in dynes per cm.
p = the perimeter in mm.

Taking 35 dynes per cm. for the surface tension and the perimeter of the respiratory apparatus alone, the equation becomes

$$\sin \alpha = \frac{v(\text{mm}^3)}{p(\text{mm})} \times \frac{0.030}{35 \times 0.102} = 0.0084 \frac{v}{p} \qquad (11)$$

where 0.030 is the difference in specific gravity between the larva and the displaced liquid (the specific gravity of the Aerosol-OT solution having a surface tension of 35 dynes per cm. was 1.001) and $\frac{v}{p}$ is the volume-perimeter ratio. The results of the computations of volume, perimeter, volume-perimeter ratio, and the angle of depression, α, are summarized in table 2.

In table 2 it is seen that the angle of depression, α, required for floating by the

respiratory apparatus alone at a liquid surface with a surface tension of 35 dynes per cm. varied from 0° 3.6′ for the first instar, through 0° 6.2′ for the second instar, to 0° 23.6′ for the fourth instar, the volumes being 0.0725, 0.1189, and 1.123 mm^3 respectively. Thus the required values of the angle of depression should be developed automatically, as shown in equation 6, on all horizontal surfaces if wetting did not occur. The eventual sinking of larvae at this surface tension of 35 dynes per cm. must have been due to lowering of the surface tension by evaporation, or possibly to some chemical action of the detergent on the surface of the respiratory apparatus, which reduced its non-wetting properties.

Since the perimeter of the pair of prepupal trumpets is of the same order of magnitude as that of the respiratory apparatus, and since the floating of larvae at surface tensions between 39 and 49 dynes per cm. involves both the respiratory apparatus and the prepupal trumpets, the volume/perimeter ratio at these surface tensions would be about half as much as that at 35 dynes per cm. The value required for floating would be about 0° 1.8′, 0° 3.1′, and 0° 11.8′ respectively, for larvae of

TABLE 2

Angles of depressions, α, required to float different stages of A. quadrimaculatus *larvae in a liquid with a surface tension of 35 dynes per cm.*

LARVAL STAGE	VOLUME (MM^3)	PERIMETER OF RESPIRATORY APPARATUS (MM)	PERIMETER OF PREPUPAL TRUMPETS (MM)	$\frac{v}{p}$ (MM^2)	α
First instar	0.0725	0.607	0.568	0.1191	0° 3.6′
Second instar	0.1189	0.925	0.934	0.216	0° 6.2′
Fourth instar	1.1230	1.375	1.140	0.817	0° 23.6′

these three developmental stages. In natural bodies of water, in which the surface tension is usually above 60 dynes per cm., and specific gravity (at 25°C) probably less than 1.0, the value of α required for floating of anopheline larvae is apparently very close to 0°. This should be even more apparent when the palmate hairs are involved.

SUMMARY AND CONCLUSIONS

A study was made to determine quantitatively the forces involved in the flotation of *A. quadrimaculatus* larvae at liquid surfaces. Since the surface tension and the interfacial tension forces are the only forces available for floating, a mathematical treatment of the forces involved in the floating of a solid at a liquid surface was presented.

Preliminary observations showed that anopheline larvae depend on their posterior respiratory apparatus for floating during respiration; on their prepupal trumpets for the floating of the anterior part during feeding; and on their palmate hairs as auxiliary floating apparatus. A surface tension of about 30 dynes per cm. is the critical value for floating the larvae, since below this value they sink. At surface tensions between 30 and 35 dynes per cm. the larvae can float by the respiratory apparatus

alone for about 15 minutes. In the range of surface tensions between 39 and 49 dynes per cm., the larvae float by both the respiratory apparatus and the prepupal trumpets. At surface tensions over 50 dynes per cm., the respiratory apparatus, the prepupal trumpets, and the palmate hairs are all involved in the floating.

From a quantitative analysis of the volume of each larva, the perimeter of the respiratory apparatus and the prepupal trumpets, and from the specific gravity of the larva, the values of the angle of depression, α, were computed for first-, second-, and fourth-instar larvae. These values showed that an angle of depression very close to 0° should be enough to float anopheline larvae at the surface of natural bodies of water. Although the contour of the surface of the respiratory apparatus is quite irregular, thus making the value of $\frac{dy}{dx}$ variable, the required values of α for floating should be developed automatically on all horizontal surfaces, if wetting does not occur. The shape of the prepupal trumpets should also permit the development of the angle of depression required for floating, provided that wetting does not occur.

From this study it is concluded that anopheline larvae require an angle of depression, α, very cloe to 0° for floating during respiration and/or feeding; that the respiratory apparatus is essential for floating during respiration, and is the most water-resistant part of the larva; that the prepupal trumpets are essential for floating during feeding, and are wetted more easily than the respiratory apparatus; and that the palmate hairs are auxiliary floating structures, and are the first of these three floating structures to become wetted when the surface tension of the water is lowered.

RESUMEN Y CONCLUSIONES

Se realizó un estudio para determinar cuantitativamente las fuerzas que permiten la flotación de *A. quadrimaculatus* en superficies líquidas. Como las fuerzas de tensión superficial e interfacial son las únicas que permiten esta flotación, se ha presentado por lo tanto un estudio matemático acerca de las fuerzas que permiten la flotación de un sólido en una superficie líquida.

Las observaciones preliminares demonstraron que las larvas anofelinas dependen de su mecanismo respiratorio posterior para flotar durante la respiración, de sus "trompetas" prepupales que les permiten flotar su parte anterior durante la alimentación y de sus filamentos palmados como aparato auxiliar para la flotación. Una tensión superficial de 12 dinas por centímetro es el valor crítico para flotar, a una tensión menor las larvas se hunden. En tensiones superficiales de 30 y 35 dinas por centímetro las larvas pueden flotar por espacio de 15 minutos usando solamente su aparato respiratorio. En tensiones de 39 a 49 dinas centímetro éstas pueden flotar usando su aparato respiratorio y sus "trompetas" prepupales a la vez. En tensiones de más de 50 dinas por centímetro, éstas hacen uso del aparato respiratorio, las "trompetas" prepupales y los filamentos palmados.

Por medio de un análisis cuantitativo del volumen de cada larva, el perímetro del aparato respiratorio y de las "trompetas" prepupales y usando el peso específico de cada larva, se pudo calcular el ángulo de depresión, α, para las fases larvarias primera, segunda y cuarta. Los resultados demostraron que un ángulo de depresión de casi

0° debe ser suficiente para flotar larvas anofelinas en la superficie de cuerpos naturales de agua. Aunque la superficie del aparato respiratorio es bastante irregular, variando así el valor de $\frac{dy}{dx}$, los valores de α requeridos para flotar deben desarrollarse automáticamente en todas las superficies horizontales, ésto es, si este ōrgano no se humedece. La forma de las "trompetas" prepupales también debe permitir el desarrollo del ángulo de depresión que requiere la flotación si éstas no se humedecen.

Se ha concluído a través de este estudio que las larvas anofelinas requieren un ángulo de depresión, α, de casi 0° para flotar durante la respiración y/o la alimentación; que el aparato respiratorio es esencial para flotar durante la respiración y es la parte de la larva más resistente al agua; que las "trompetas" prepupales son esenciales para flotar durante la alimentación y se humedecen más fáclimente que el aparato respiratorio y que los filamentos palmados son estructuras auxiliares para flotar y son las primeras en humedecerse cuando se reduce la tensión superficial del agua.

THE DECLINE AND LAST RECORDED OUTBREAKS OF MALARIA IN NORTH CAROLINA

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During the past decade the incidence of malaria in North Carolina has manifested a steady and marked decline, a trend which conforms to the general pattern of malaria infection in the United States (Andrews 1948, Andrews *et al.*, 1950). Prior to 1940 the prevalence of the disease in the eastern half of North Carolina was of sufficient magnitude to constitute a definite hindrance to the health and economic growth of that section of the state. The severity of the problem in localized areas, however, was not accurately established until after 1937 when the State Board of Health organized a Malaria Investigation and Control Unit. To determine the prevalence of the disease in the malarious counties, this unit conducted periodic, county-wide blood slide surveys among school children through the first six grades. By this method malaria was shown to be sporadic in its distribution, the disease frequently being absent in one portion of a county although of high endemicity in another section. For example, White (1944) in discussing this aspect, reported that of the 45 schools in Edgecombe County, the county which had the highest incidence of malaria in 1937, the infection rate in the children was negative in 10 schools and above 15 per cent in five.

The data for these grade school surveys for the period 1937 through 1943 clearly portray the decline and almost complete disappearance of the disease from former problem areas (Table 1). Seven schools in which the children exhibited infection rates of 9 to 62 per cent in 1937–38, showed a drastic reduction in the number of positive films in the 1942–43 surveys. Only one positive slide was reported from these schools, the rate of parasitemia for the 1942–43 period being 0.1 per cent as compared to 17.0 per cent in 1937–38. All schools, regardless of the race involved or geographic location, manifested the same precipitous drop in the rate of infection. Inasmuch as the problem areas listed in Table 1 lay beyond the scope of military or war industrial establishments where anti-anopheline measures were in effect, the disappearance of malaria from these areas cannot be attributed to the extensive control efforts practiced by State and Federal health agencies during the war period. This fact makes it difficult to appraise the exact role such suppressive measures played in the over-all decline of the disease throughout the state. Prior to 1941 considerable drainage was accomplished in many of the malarious counties by the combined efforts of Federal, State, and local agencies. This activity, the intensive promotion of larvicidal programs by local health officials, the rise in rural living standards, the increased number of mosquito-proofed homes, the greater use of household sprays, the modification of agricultural practices, and the availability of therapeutic agents can all justifiably be considered as factors contributing to the gradual decline of malaria incidence.

With the return of infected military personnel in 1946 and 1947 the rate of positive blood films among school children still remained low. The impact of the infected veterans, however, was reflected in the increased number of positive smears recorded by the North Carolina State Laboratory of Hygiene in the routine examination of submitted blood films. The prevention of any augmentation of the disease in the civilian population exposed to these new sources of infection in areas where *Anopheles quadrimaculatus* Say prevailed, may be ascribed, at least in part, to the effectiveness of the Extended Residual Spray Program which operated during 1946–47. This

TABLE 1

Results of thick film blood surveys in North Carolina schools for 1937–38 as compared to those for 1942–43

COUNTY AND SCHOOL	RACE	1937–38			1943		
		No. examined	No. positive	Per cent positive	No. examined	No. positive	Per cent positive
Robeson County							
Harper's Ferry	I	29	18	62.1	49	0	0.0
Whitehill	I	65	6	9.2	46	0	0.0
Marietta	C	82	7	8.5	89	0	0.0
South Robeson	W	122	13	10.6	150	0	0.0
Bladen County							
Kelly	W	195	48	24.6	76	1	1.3
Wayne County							
Brodgen	W	196	18	9.2	271*	0	0.0
Edgecombe County							
Battleboro	C	136	11	8.1	39	2	5.1
Coker	C	79	5	6.3	46	0	0.0
Chinquapin	C	57	5	8.8	41	0	0.0
Greene	C	92	3	3.3	45	0	0.0
Keech	C	59	4	6.8	29	0	0.0
Mark's Chapel	C	54	5	9.3	50	0	0.0
Pittman's Grove	C	62	15	24.2	20	0	0.0
Wimberly	C	63	11	17.5	56	0	0.0
Leggett	W	155	9	5.8	186	1	0.5
Halifax County							
Log's Chapel	C	24	2	8.2	23	0	0
Total		1470	180	12.2	1216	4	0.3

* Survey conducted in 1942. I = Indian, C = Colored, W = White.

program, a cooperative undertaking by the Communicable Disease Center and 13 southeastern states, consisted of residual spraying of homes with 5 per cent DDT and was designed specifically to combat malaria infections introduced by returning veterans. The Extended Program was succeeded by a five-year Malaria Eradication Program (Andrews and Gilbertson, 1948) conducted upon the same control principle of residual house treatments. Coincident with these extensive efforts for 1946 through 1950 there has been a continued and pronounced reduction of malaria infection throughout the endemic areas of the eastern seaboard of North Carolina.

In retrospect, with the incidence of malaria almost to the vanishing point in North

Carolina, the question arises as to when the last outbreaks of the disease occurred within the state. Since the state and national trends of the disease parallel, it is likewise appropriate to consider the same issue for the United States. The last recorded epidemics of any extent in this country since 1942 are those reported in 1943 and 1944 for the Santee Cooper Reservoir in South Carolina (Anon, 1945), where in certain areas adjacent to the impoundment, blood film surveys revealed infection rates of 38.3 per cent and 38.7 per cent, respectively, in population samples of 1,352 and 470 individuals. Aside from these outbreaks, records of confirmed malaria transmission are limited to endemic infections in the Santee Cooper area in 1942 and 1943 (Anon, 1945), a small endemic focus in Louisiana, Missouri in 1942 described by Brooke (undated), and the occurrence of two primary cases in Josephine County, Oregon in 1944 (Osgood, 1945). Frohne *et al.* (1950) present data of gradually declining monthly infection rates in a population sample of 600 Negroes in the Santee Cooper Reservoir area for 1945, 1946, 1947, and 1948. The rates of 5.1 to 9.4 per cent in the summer of 1945 presumably reflect anopheline transmission in the area but the decreased magnitude of infection in the years 1946 through 1948, in all probability, is more indicative of relapses than of primary infections. Quinby (1950) states that of 180 laboratory confirmed cases in Alabama, Georgia, Mississippi, and South Carolina in 1948 only 59 were believed to have originated in this country. Andrews *et al.* (1950) report that appraisal of 55 confirmed cases in Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Texas in 1949 revealed only 19 to be primary indigenous infections.

That such a paucity of information exists on this phase of malariology in the United States possibly stems from the fact that during the past decade, particularly before 1945, relatively few individuals realized the pronounced downward trend of malaria incidence in this country. Minor epidemics in light of the outbreaks of the years 1930 to 1940 appeared inconsequential and hardly worthy of documentation. Such appraisal is applicable to the epidemics treated in this article. At the time of the reported observations in 1943, little consideration was given to the importance of these findings in relation to the general trend of malaria prevalence. In time, with the absence of further epidemics and the rapid disappearance of endemic foci, the data assumed definite significance since they apparently represented the last recorded outbreaks of malaria in the state.

In North Carolina the records of the State Laboratory of Hygiene concerning the routine examination of blood films submitted by physicians show positive parasitemia for 25, 13, 21, 10, 14, and 16 smears during the years 1940–45, followed by an abrupt rise to 58 and 33 confirmations in 1946 and 1947. In 1948 only 3 positive smears were reported and for 1949 the findings have been entirely negative. In the special school surveys the number of positive blood films progressively diminished during the period 1940–45. The Nash County survey in 1945 showed nine positives in the 5,619 slides taken. Subsequent to 1945 no positive smears have been recorded, the negative results leading to a curtailment and then to cessation of this type of malaria surveillance in 1950. The lack of epidemiologic appraisal on the routine laboratory confirmation renders impossible any decision as to source of infection for the positive cases which are listed for the years 1943 through 1948. Andrews *et al.* (1950) record

only two confirmed malaria cases from North Carolina in 1949, appraising one as primary (Anson County), the second as a relapse.

The purpose of this presentation is twofold: (a) To document the two malaria epidemics in North Carolina which constitute the last recorded outbreaks in the state and which presumably represent two of the most recent in the United States. (b) To stimulate the documentation of similar outbreaks which may have occurred in other states. The recording of such data is essential to what is hoped to be the final obituary on malaria as an important disease in this country.

The 1943 epidemics at Shocco Springs and Chub Lake occurred in counties which lie along the northern border of North Carolina (figure 1). Both areas are located

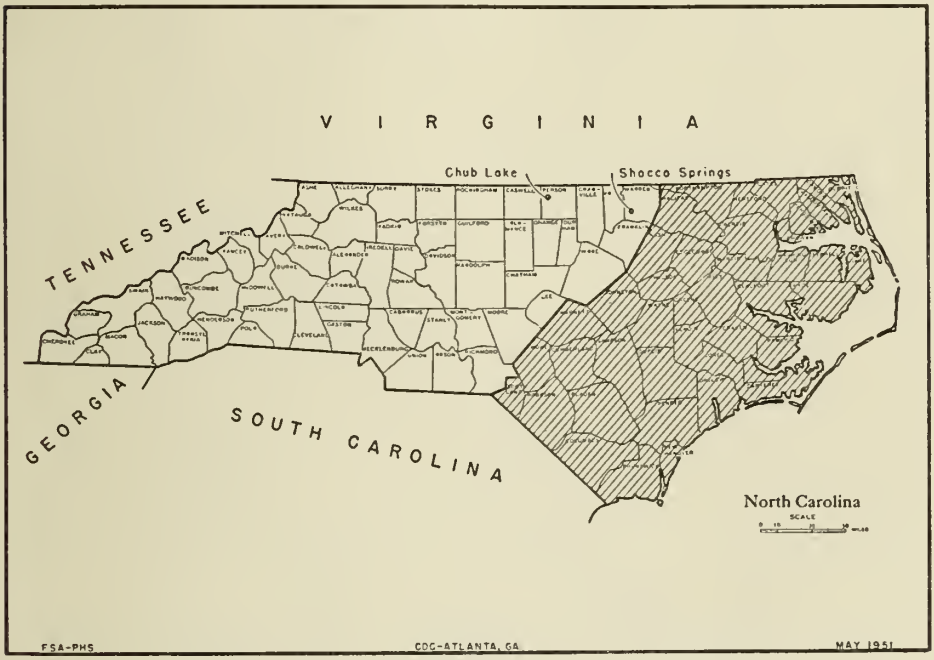

FIG. 1. Map of North Carolina showing malarious section of state (shaded portion) and location of 1943 epidemics in Warren and Person Counties.

in the Piedmont Region and are generally considered as being outside the normal malarious portion of the state.

SHOCCO SPRINGS EPIDEMIC

Description: Shocco Springs is located in the southwestern corner of Warren County (figure 1). During the Civil War period this community was a thriving health resort, renowned for the curative effects of its mineral springs. Since the abandonment of the resort in the latter part of the 19th century, the community returned to agricultural pursuits, and today the major portion of the area is farmed by small landowners and tenants. Aprpoximately 245 individuals inhabit this section of Warren County, which according to the 1940 census, had a total population of 23,145.

In general the land is relatively unproductive and a rather low standard of living prevails.

The principal source of mosquito breeding in the community is a large, shallow, natural pond, Old Duck Pond, which receives water from Shocco Springs and Shocco

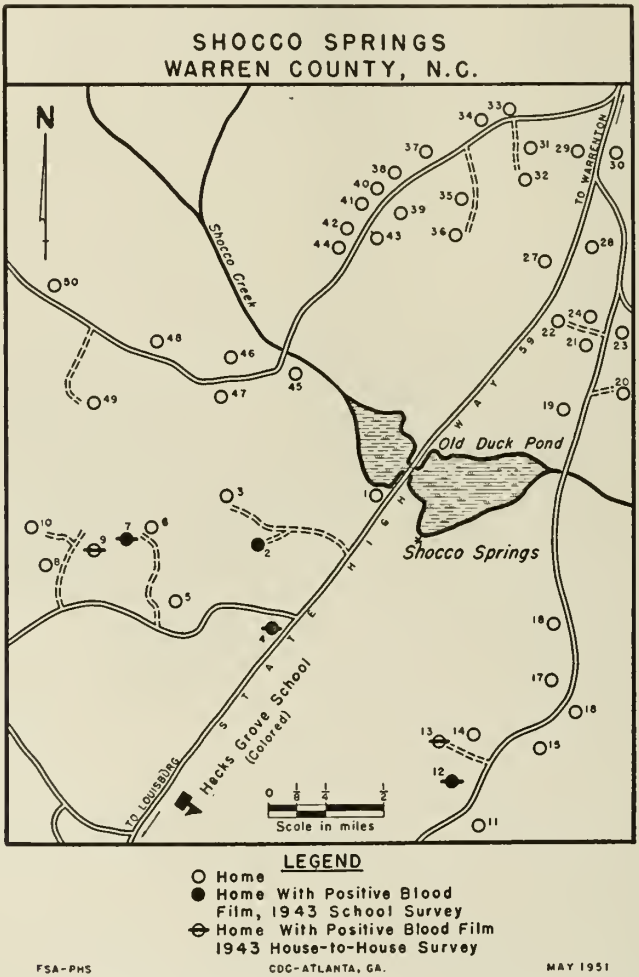

FIG. 2. Map showing the Shocco Springs Community and the homes with individuals having positive blood films on the 1943 surveys.

Creek, the latter draining the greater part of the area (figure 2). The pond is completely overgrown by various aquatic plants, with alder bush and willow invading the marginal zones.

Epidemiologic history: In August, 1943, a Negro physician requested assistance from the North Carolina State Board of Health in the diagnosis of a disease prevalent in the Shocco Springs area. The infection manifested some symptoms of typhoid fever

but the entire community had been immunized against this disease during the same year. The clinical impression of the State Epidemiologist, however, indicated that the disease probably was malaria. Blood films taken from two hospitalized patients confirmed this impression.

Prior to 1943 little authentic information is available on the incidence of malaria in Shocco Springs. As no organized health department existed in the county, the only data of possible significance relate to the mortality records maintained by the State Board of Health. During the period 1924 through 1939, three deaths ascribed to malaria occurred in Warren County. Since 1939, no deaths from this disease have been recorded. Physicians serving this southern sector of Warren County reported the occurrence of some malaria in 1943, but stated that the disease had not been a local problem since the epidemic of 1921, which centered around Lake Largo, an area approximately one and one-half miles north of Shocco Springs.

Malaria blood slide and household surveys: To determine the distribution and intensity of infection in the Shocco Springs community, thick film blood surveys were conducted in an area within one mile of the Old Duck Pond (figure 2) in August and October, 1943.

The August survey was confined to the southern section of the community, slides being taken in houses 1 to 4, 7, 9, 12, and 13 (figure 2). Of the 61 individuals sampled, 54 were Negroes. Twenty-four films disclosed parasitemia with *Plasmodium falciparum*, all positives occurring in the Negro population, thus yielding an infection rate of 39.3 per cent in the total individuals examined (or 44.4 per cent in the Negro sample alone). The oldest infected individual was 44 years of age, the youngest 3 years. In the families living in houses numbered 4 and 12 (figure 2) six and seven individuals, respectively, were shown to be infected.

The October survey was made in the Hecks Grove School which served the majority of the Negro children living within the Shocco Springs area. Of the 85 individuals examined, 9 gave positive smears for *P. falciparum*, an infection rate of 10.6 per cent. Three of these nine children represented individuals which had yielded positive smears on the August survey, the remaining six had not been tested previously.

In February, 1944, a detailed reconnaissance was effected in Shocco Springs to locate the homes, determine the racial composition of the population, interview the inhabitants as to their malaria history, and to classify the types of dwellings. Of the 245 individuals living in the community, 57.1 per cent were Negro, 42.9 per cent were white. Twenty-seven per cent of the white population and 42 per cent of the Negro gave histories of malaria infection. Consultation with family physicians verified all but one of these reports of clinical activity. Only 22 homes or 53 per cent showed any type of mosquito proofing, over 30 per cent of the houses being in need of major repair.

Entomologic surveys: Inspection of a number of homes in August, 1943 revealed adult female *A. quadrimaculatus*, as many as 10 specimens being captured in individual houses. Larval surveys in 1944 showed the occurrence of *A. quadrimaculatus* in the Old Duck Pond.

DISCUSSION

Despite the explosive nature of the 1943 epidemic, the disease apparently disappeared from the community in 1944. Household contacts with the families positive for malaria in 1943 and with their physician indicated that the disease no longer was of any consequence in the community. Although therapeutic treatment had been administered in the area, it is difficult to ascribe the rapid disappearance of the disease to this agent alone. Yearly contacts have been maintained with the community, but to date no cases of malaria have been noted since the 1943 outbreak.

It is conjectural as to the original source of infection in the community. Popular opinion agreed that the disease was introduced into Shocco Springs by one family which arrived in the fall of 1942 (House No. 1), and had previously lived in a malarious area of an adjacent county. However, available evidence neither proved nor disproved this hearsay.

CHUB LAKE EPIDEMIC

Chub Lake is an impoundment located in the northwest section of Person County approximately four miles from Roxboro (figure 3). This lake serves chiefly for recreational purposes. Within a one-mile area of Chub Lake the resident population totals 303 individuals, approximately one per cent of the aggregate county population of 23,145. The principal occupations of the inhabitants are farming and employment in nearby textile mills. Although all dwellings shown on the map are within the flight range of the *A. quadrimaculatus*, the focus of malaria cases centered along the southeast shore of the lake (Houses 1 to 13).

The impoundment covers an area of approximately 50 acres and is bordered along the entire northern, eastern, and western shores by a two to three foot strip of aquatic vegetation. At the southern tip of the lake a narrow peninsula protrudes into the impoundment, thereby confining the flow to a channel 30 to 40 feet in width. Behind the peninsula lies a shallow marshy expanse which is densely covered with several species of marsh smartweed, *Persicaria portoricensis*, *P. muhlenbergia*, *P. hydropiperoides*, and golden club, *Orontium aquaticum*. In addition, small patches of water lilies, *Castalia odorata*, are scattered over the water surface. The marsh smartweeds also extend into the lake where they form irregular islands of vegetation.

Epidemiologic history: In August, 1943, the North Carolina State Board of Health was requested by the District Health Officer to make a survey of a reported outbreak of malaria in the Chub Lake area of Person County. Positive blood films from several inhabitants confirmed the clinical diagnosis of malaria.

A review of the malaria mortality records for Person County revealed no deaths subsequent to 1927. Available morbidity data were too unreliable and incomplete to accurately portray the pre-epidemic incidence of malaria in the Chub Lake community, or in the county. However, interviews with Person County Health Department personnel and inhabitants in the vicinity of Chub Lake indicated that malaria probably had been present in that section for five years preceding the 1943 outbreak. According to information from local physicians, 28 residents of the Chub Lake community received treatment for the disease in the summer of 1943.

Malaria blood slide and household surveys: In September 1943, a house-to-house

blood film survey was conducted in the isolated hill community at the southern tip of the impoundment. From all reports, this localized area (fig. 3 Houses 1 to 12) was the principal seat of malarial infection in the Chub Lake section. Smears were taken from 24 white inhabitants, and seven of the blood films proved to be positive

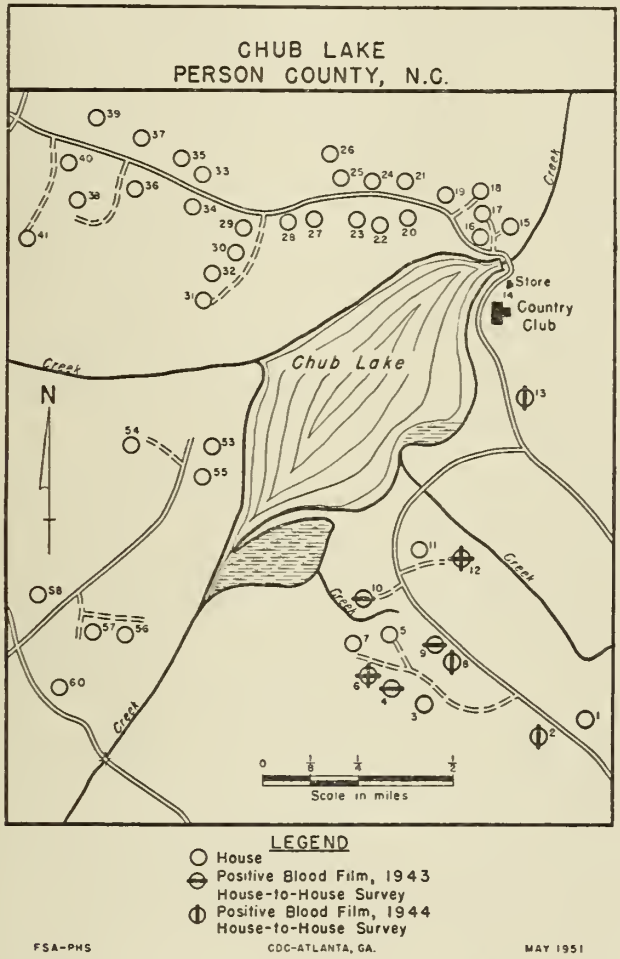

FIG. 3. Map showing Chub Lake Community and the homes with individuals having positive blood films on the 1943 and 1944 surveys.

for *Plasmodium vivax*, an infection rate of 29.2 per cent. Three of the infections were in children 3 to 6 years of age, the remainder coming from individuals 12 to 51 years of age. Although 58 per cent of the population sampled was receiving medication at the time of examination, six of the seven positive films came from persons under treatment. On questioning, four individuals revealed no history of malaria, five reported fever in 1942, one in 1938, and the remaining 14 indicated 1943 as being the first year they had noticed any symptoms of the disease. Two of the positive films came from individuals with a history of malaria in 1942.

In late August 1944, an extensive house-to-house survey covered all homes within a one-mile radius of Chub Lake. Of the 303 slides taken, six, or 1.9 per cent, showed the presence of *Plasmodium vivax*. With the exception of the case in House No. 13, all infected individuals lived in the same hill community in which the 1943 outbreak occurred (fig. 3, Houses 2, 6, 8, and 11). None of these persons had been sampled on the 1943 survey. Three of the individuals were children of the ages 1, 4, and 5 years, the infection in the one-year-old child, in all probability, representing anopheline transmission in 1944.

Entomologic surveys: Adult and larval anopheline surveys were conducted in the immediate vicinity of Chub Lake in September 1943, primary attention being given to the southern portion of the impoundment where the focus of malaria existed. Examination of pig pens, mule stables, and cow sheds in this section revealed the presence of adult *Anopheles punctipennis*, *A. walkeri* and *A. quadrimaculatus*. The latter species, although predominant, occurred in relatively low densities, only two shelters contained more than 15 specimens (21 and 34 females respectively). In view of the proximity of breeding sources, these low densities of adult *A. quadrimaculatus* in animal sheds were surprising. Also of interest were the data from several house inspections which revealed the number of adult mosquitoes in dwelling number 10 to exceed the majority of those found in outdoor shelters, 15 female *A. quadrimaculatus* were captured in one bedroom. A similar relationship between indoor and outdoor counts was manifested in the July, 1944 inspection when a total of 19 female *A. quadrimaculatus* was taken in the same house (No. 10) as on the 1943 survey, whereas maximum counts in adjacent animal shelters did not exceed six in number. These findings conflict with an opinion prevalent within the state that high outdoor densities of A. *quadrimaculatus* are prerequisite to any appreciable invasion of the home by the mosquito. In the more malarious coastal areas of North Carolina, adult densities in dwellings rarely approach 10 in number, even when outdoor counts of the mosquito are in the range of 1,000 to 1,500 adults per animal shelter. From the limited survey data available, it is suggestive that in their feeding habits the mosquitoes of the Chub Lake area may be more anthrophilic than zoophilic.

As a basis for formulating control recommendations, larval surveys were made in Chub Lake and the nearby water courses. Results indicated that the principal source of *A. quadrimaculatus* was at the southern end of Chub Lake where moderate breeding occurred in the dense patches of marsh smartweeds and golden club.

Discussion

After the 1943 surveys, specific recommendations for controlling the breeding of *A. quadrimaculatus* in Chub Lake were submitted to the owner of the impoundment. However, other than a lowering of the water level of the lake in 1944, no effective remedial measures were invoked. As the 1944 entomologic survey showed, *A. quadrimaculatus* continued to breed in the impoundment and the adult mosquitoes invaded the nearby dwellings. From the blood slide survey data, it seems evident that some transmission occurred in 1944 but the level of infection appeared to be lower than that prevailing in 1943. Subsequent to 1944, household interviews in the area revealed no reported malaria. In 1946 and 1948, all dwellings in the area received residual

treatment with five per cent DDT. To date, there has not been any further evidence of malaria infection in the Chub Lake section.

SUMMARY AND CONCLUSIONS

1. The decline in the incidence of malaria in North Carolina during the period 1937 to 1950 is reviewed. Blood film data from schools surveyed in 1937–38 and again in 1942–43 are presented to show the drastic reduction in malaria infection from 12.2 per cent to 0.3 per cent in areas where no organized control programs were in operation. Records of the State Laboratory of Hygiene likewise reflect the same general downward trend. Positive blood smears submitted by physicians averaged 16 confirmations per year for the period 1940–45. During 1946–47, the return of infected military personnel from foreign countries led to a sharp increase in the number of positive blood films. An abrupt decrease in positive smears followed, three being listed for 1948, and none for 1949 or 1950.

2. Available information in the literature indicates little evidence of malaria transmission in the United States subsequent to 1945. The last recorded epidemics are those in the vicinity of the Santee Cooper Reservoir in South Carolina in 1943 and 1944.

3. Detailed reports are presented for the last two malaria outbreaks in North Carolina which occurred at Shocco Springs (Warren County) and Chub Lake (Person County) in 1943.

4. In the Shocco Springs area a thick film survey of 61 individuals in August, 1943 yielded an infection rate of 44.4 per cent in the 54 Negroes examined. An October survey in the Negro school serving this community showed an infection rate of 10.6 per cent in the 85 children tested. All infections were due to *Plasmodium falciparum.* Although only therapeutic measures were implemented, the disease apparently disappeared from the community in 1944.

5. At Chub Lake, North Carolina, a house-to-house blood film survey in September, 1943 disclosed a parasitemia rate of 29.2 per cent in the 24 white individuals examined. A similar survey in August, 1944 of the 303 individuals living within one mile of the impoundment yielded an infection rate of 1.9 per cent, all of the positive smears being taken from individuals residing in the same general locality covered on the 1943 survey. Three of the positive smears found were from children of the ages 1, 4, and 5 years, the infection in the one-year-old child undoubtedly represented anopheline transmission in 1944. *Plasmodium vivax* was the causative agent of all infections in the Chub Lake community. Despite the lack of adequate control measures in 1945, the disease did not reappear in the area.

6. Limited house surveys in the Chub Lake area showed the densities of *Anopheles quadrimaculatus* inside dwellings occasionally to exceed those encountered in outdoor resting sites. The general level of *A. quadrimaculatus* prevalence in the area was low, counts above 15 mosquitoes per animal shelter rarely being observed.

LITERATURE CITED

Andrews, J. M. 1948. What's happening to malaria in the U. S. A.? Amer. Jour. Pub. Heal., **38:** 931–942.

Andrews, J. M. and W. E. Gilbertson 1948. Blueprint for malaria eradication in the United States. Jour. Nat. Mal. Soc., **7**: 167–170.

Andrews, J. M., Quinby, G. E., and A. D. Langmuir 1950. Malaria eradication in the United States. Amer. Jour. Pub. Heal., **40**: 1405–1411.

Anonymous 1945. A malaria survey of the Santee-Cooper Reservoir, South Carolina. Abbrev. Rpt. South Carolina State Board of Health and Malaria Control War Areas, Public Health Service: 1–20.

Brooke, M. M. Undated. Report on malaria survey along the upper Mississippi River, Parts I (1940–41) and II 1942, Office Interstate Malaria Survey Board of State Health Commissioners. Upper Mississippi River Basin pp. 1–80.

Frohne, W. C., Weathersbee, A. A., Williams, G. M., and J. W. Heart. 1950. Observations of the persistence of *Plasmodium* infections in *Anopheles* mosquitoes in an area of low observed human malaria parasitemia in South Carolina. Jour. Nat. Mal. Soc., **9**: 10–24.

Osgood, S. B. 1945. Malaria and the returning soldier. Jour. Amer. Med. Assoc., **128**: 512–513.

Quinby, G. E. 1950. Epidemiological appraisal of reported malaria in the United States from 1947 through 1949. Jour. Nat. Mal. Soc., **9**: 397–400.

White, C. M. 1944. North Carolina Malaria Bloodslide Survey. Malaria Control War Areas, Public Health Service, November Field Bull.: 1–3.

RESUMEN Y CONCLUSIONES

1. Se ha analizado la reducción en la incidencia de malaria en Carolina del Norte durante el período de 1937 a 1950. Preséntanse los resultados obtenidos a través de estudios de laminillas de sangre en las escuelas durante los períodos de 1937–38 y 1942–43 para demostrar la reducción drástica de la infección malárica de 12.2 por ciento a 0.3 por ciento en áreas donde no habían operado organizados programas de control. Los informes del Laboratorio Estadual de Hygiene también demuestran este descenso general. Laminillas de sangre positivas sometidas por médicos alcanzaron un promedio de 16 confirmaciones por año durante el período 1940–45. Durante 1946–47, el regreso de personal militar infectado de países extranjeros dió origen a un gran aumento en el número de laminillas de sangre positivas. Éste fué seguido por un descenso repentino en el número de laminillas positivas, se reportaron tres para 1948 y ninguna para 1949 o 1950.

2. La información disponible indica que es poca la evidencia de malaria en los E.E.U.U. después de 1945. Las últimas epidemias registradas son aquellas en la vecindad del "Santee River Reservoir" en Carolina de Sur en 1943 y 1944.

3. Preséntanse reportes detallados de los dos últimos brotes de malaria en Carolina del Norte que occurrieron en "Shocco Springs (Warren County)" y en "Chub Lake (Person County)" en 1943.

4. Un estudio de laminillas de sangre densas en 61 individuos procedentes del área de "Shocco Springs" en agosto 1943 produjo una proporción de infección de 44.4 por ciento en los 54 negros examinados. Un estudio llevado a cabo en octubre en la Escuela para Negros de esta comunidad demonstró una proporción de infección de 10.6 por ciento en los 85 niños examinados. Todas las infecciones fueron causadas por *Plasmodium falciparum*. Aunque solamente se usaron medidas terapéuticas la enfermedad parece haber desaparecido de esta comunidad en 1944.

5. Un estudio de laminillas de sangre efectuado de casa a casa en septiembre, 1943 en "Chub Lake," Carolina de Norte, reveló una parasitemia de 29.2 por ciento en los 24 individuous blancos examinados. Un estudio similar en agosto, 1944 en 303

individuos que residían a una milla del lago produjo una proporción de infección de 1.9 por ciento, todas las laminillas positivas procediendo de la misma localidad general cubierta por el estudio de 1943. Tres de las laminillas positivas ocurrieron en niños de 1, 4 y 5 años de edad, la primera de éstas indudablemente fué representativa de transmisión anofelina en 1944. *Plasmodium vivax* fué el vector causante de todas las infecciones en la comunidad de "Chub Lake." A pesar de la falta de medidas de control adecuadas la enfermedad no reapereció en el área en 1945.

6. Estudios limitados en viviendas en el area de "Chub Lake" demonstraron en éstas densidades de *Anopheles quadrimaculatus* que a veces excedieron las encontradas afuera. El nivel general de la preponderancia de *A. quadrimaculatus* en el área fué bajo, muy raramente se registraron cantidades de más de 15 mosquitos por cada vivienda animal.

FACTORS INFLUENCING THE SEARCH FOR ANOPHELINE LARVAE IN SARDINIA[1]

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The progress of the ERLAAS project for the eradication of *Anopheles l. labranchiae* in Sardinia has been reviewed by Logan, 1950. Once the great bulk of the mosquitoes had been destroyed, the problem arose of finding those that remained so that efforts could be concentrated on their destruction. I was invited to participate in the work of finding these last mosquitoes on the island during the 1950 breeding season.

The fact that *Anopheles l. labranchiae* is a species indigenous to Sardinia presented a situation very different from that encountered in the successful eradication campaigns which had previously been waged against the invading *Anopheles gambiae* in Brazil (Soper and Wilson, 1943) and in Egypt (Shousha, 1948). Despite the most painstaking scouting of all known water surfaces for anopheline larvae, as well as searches of possible adult resting places, *A. l. labranchiae* continued to be found occasionally in areas that had been thought to be clean for as much as, or in some cases more than, two years. The present account deals with studies of anopheline larval behavior in Sardinia, and how this behavior may affect the ability of the entomologist to detect larvae when they are present in only small numbers.

The conventional method of searching for anopheline larvae is that of using a dipper or net to skim up portions of the water surface and examining this surface water against the convenient white background of an enamel pan or dish. A multitude of variations of this method have been reported. In Sardinia during the 1950 season dippers of the sort described and figured by Russell, West and Manwell, 1946, were used. These dippers have a diameter of 15 cm. and a capacity of about 400 cc. To measure the total larval population of limited areas other methods have been described, such as that of fencing off sections of water surface and thoroughly dipping the enclosed water from which the larvae cannot escape by lateral movements (Bates, 1941). When the first method is used, the surface of a large area may be sampled; when the second method is used, the total population may be determined, but only for a small fraction of the entire area of the presumed breeding place. The experience in Sardinia indicated the need to stress that both these methods only sample the population of a breeding area; neither method is adequate for determining the absolute presence or absence of larvae in an extensive breeding place when the populations are very low. This is a point generally overlooked by writers who report a "100 per cent kill" of larvae when they have dipped in an area and failed to find larvae following field tests of larvicides.

[1] The studies on which this paper is based were conducted with the support and under the auspices of the Division of Medicine and Public Health of The Rockefeller Foundation with the co-operation of the Italian government as part of the work of ERLAAS (Ente Regionale per la Lotta Anti-Anophelica in Sardegna).

The difficulty of finding larvae, and an expression of how great the element of chance in such finds is, when population levels are low, was well illustrated by an area in the valley of the Riu Mulargia, Sardinia, found positive for *A. l. labranchiae* during the first week of August 1950. Scouting of this isolated river valley had been negative during 1949 and through July 1950. During the first week of August scouts found larvae of *labranchiae* in two small pools in the partially dried up stream-bed. A subsequent intensive search of these pools produced nine fourth-instar and one third-instar larvae. The scouts concentrated their search in the area but found no additional larvae. At a point about 100 meters from one of the positive pools was a gully beside the main stream course with a strip of water 40 meters long, two meters wide, and a half meter deep, richly provided with submerged and surface vegetation and a moderate amount of emergent vegetation (figures 1 and 2). The water was still and fully exposed to the sun, an ideal *labranchiae* habitat. The scouts working the area had found this pool to be negative for *labranchiae*, although it supported a heavy culicine population.

I started dipping at one end of this pool, and on the fourth dip a third-instar larva of *labranchiae* was found along with numerous culicine larvae. Additional dipping, until 50 dips had been made within one meter of where the first larva had been taken, failed to produce any more larvae of *labranchiae*. At this point 15 scouts were placed around this end of the pool shoulder to shoulder and they proceeded to cover the restricted area of about four by two meters. Observation of these men showed that each one made an average of 12 dips per five minutes. It was 10 minutes later, and after approximately another 360 dips, that the first *labranchiae* larva was taken by these scouts. Continued dipping for one hour in the same restricted area of eight square meters produced an additional 19 second- and third-instar larvae. No larvae were found in subsequent intensive dipping of the remaining 36-meter length of the strip of water (figure 2).

Thus in a restricted area, where there were not less than 21 larvae in eight square meters of water, it took about one-thirtieth of a man-hour to find the first larva, but it took two and a half man-hours to find the second; that is, it took 75 times as much effort to find the second larva as it did the first. The impossibility of finding these larvae, using this method, by anything but chance is clearly demonstrated.

Another instructive instance of how difficult it may be to find larvae, when populations are low, was the case of a positive finding reported from a swamp, the Palude Salone in the northeastern corner of Sardinia, which I had the opportunity to investigate with and through the kindness of Dr. Thomas H. G. Aitken. This swamp was several acres in extent, in part overgrown with the tall sedge *Juncus* and emergent grasses and in part open water with heavy mats of surface vegetation. It had been repeatedly found positive for *labranchiae* and treated with larvicide during the years prior to 1950. Scouts reported the finding of larvae there in mid-May 1950. A morning search by Dr. Aitken and myself several days after the report of the positive finding failed to produce any larvae. The following day Dr. Aitken had a group of 30 scouts investigate the area thoroughly under his supervision. The men were placed in a line extending across the swamp about two meters from each other and started to dip at 10:00 A.M. following a systematic pattern across the swamp (figure

FIG. 1. Valley of the Riu Mulargia, north of Donigala, Sardinia. The gully in the foreground forms part of the partially dried up course of the stream. The group of men are clustered about a pool in which larvae of *Anopheles l. labranchiae* were found on 2 August 1950.

FIG. 2. Closeup of a pool positive for larvae of *Anopheles l. labranchiae* in a gully beside the main course of the Riu Mulargia. The scouts are engaged in intensive dipping of the strip of water two meters wide and 40 meters long.

FIG. 3. The Palude Salone, east of Arzachena in northeastern Sardinia. Approximately 17,000 dips were made by 30 scouts before the first larva of *Anopheles l. labranchiae* was found in this swamp.

FIG. 4. A spring-fed pool habitat of *Anopheles claviger* in the bed of the Riu Sa Murta Uci in southeastern Sardinia. This was one of the habitats in which intensive searching of 50 dips per square meter was carried out.

3). At 3:00 P.M. the first larva was found after four hours of dipping (one hour was taken out for lunch). The place where the larva was taken was about one-third of the way across the swamp in water about half meter deep and covered with surface vegetation. This place was in no apparent way different from the remainder of the open area of the swamp. Concentrating half the scouts in the area where the first larva had been taken produced about 40 larvae from first- to fourth-instar during the next hour. The larvae were taken in two groups about 15 meters apart. No larvae were taken elsewhere.

Allowing the usual rate of 12 dips per five minutes per man, some 17,280 dips would have been made before the first larva was found in this swamp, which was known to be positive. Here again was a striking demonstration of the capricious distribution of very small larval populations which may be located only by chance using present scouting methods.

Field experience of the sort outlined above indicated the desirability of more detailed studies of the effectiveness of dipping as a means of finding anopheline larvae. As a consequence of the ERLAAS campaign, *Anopheles l. labranchiae* was so rare that it was not feasible to work with this species. Habitats of *Anopheles claviger*, a species present in modest numbers, were therefore chosen for further study. What appeared to be favorable breeding places of this species, clear, cool, gently running water with some surface and emergent vegetation, were selected, and an area of one square meter was marked off. A dipper of the sort described above was used. Figure 4 illustrates one such habitat. Fifty dips were made in each sample plot, chosen as a possible breeding place on the basis of its favorable ecological features, and after consulting the ERLAAS records to establish that the area had not received larvicidal treatment for a year or more. The protocols of five such field trials, which proved positive for *claviger* larvae, are given in table 1. In these trials an average of two dips were made per minute and about 25 minutes devoted to making the 50 dips arbitrarily chosen as the number to cover each one square meter plot. This obviously represents the most intensive sort of search, yet it is evident from the scattered appearance of the larvae that had the number of dips been extended to a hundred or more, and even an hour devoted to each square meter, it might still have been possible to find additional larvae. These field trials raised the question of how it was possible to cover so restricted an area so thoroughly and still continue to find larvae.

In dipping for anopheline larvae at or near the water surface it is generally presumed that the larvae are at or near that surface. This is known to vary with different species, however. It was observed that larvae of *Anopheles claviger* would often remain at the bottom of the dipper sometimes wholly or partially concealed in the debris scooped up with them. Bates (1949) has described behavior of this sort as "alarm reactions" of larvae and points out that larvae may remain at the bottom in a rigid "death-feigning" position to which psychologists have applied the term "letisimulation."

As a result of this field observation it seemed desirable to establish the pattern of how long larvae avoided the surface when disturbed by the mechanical agitation of the water surface by dipping. At various times it was possible to obtain larvae of four species of *Anopheles* as follows: *A. claviger*, *A. hispaniola*, *A. algeriensis* and *A. l.*

TABLE 1

The occurrence of larvae of Anopheles claviger *in one square meter plots in the course of 50 dips*

DIP NUMBER	1ST TRIAL NUMBER OF LARVAE	2ND TRIAL NUMBER OF LARVAE	3RD TRIAL NUMBER OF LARVAE	4TH TRIAL NUMBER OF LARVAE	5TH TRIAL NUMBER OF LARVAE
1st	1 (3rd)*	0	0	1 (4th)	0
2nd	0	0	0	0	0
3rd	0	1 (1st)	1 (2nd)	0	0
4th	0	0	0	0	0
5th	0	0	0	0	0
6th	0	0	0	0	1 (3rd)
7th	0	0	0	0	0
8th	0	0	0	1 (4th)	0
9th	1 (3rd)	0	0	0	0
10th	0	0	0	1 (3rd)	0
11th	0	0	0	0	0
12th	1 (3rd)	14 (1st)	0	0	0
13th	1 (4th)	4 (1st)	0	0	0
14th	0	0	0	0	0
15th	0	0	0	0	0
16th	0	1 (1st)	0	1 (3rd)	0
17th	0	0	0	0	0
18th	0	0	0	0	0
19th	0	0	0	0	0
20th	0	0	0	0	0
21st	0	0	0	0	0
22nd	0	0	0	0	0
23rd	1 (3rd)	0	0	0	0
24th	0	0	0	0	0
25th	0	1 (1st)	1 (3rd)	0	0
26th	0	1 (1st)	0	0	0
27th	0	0	0	0	0
28th	0	0	0	0	0
29th	0	0	0	0	0
30th	0	0	0	0	1 (3rd)
31st	0	0	0	0	0
32nd	0	0	0	0	0
33rd	0	0	0	0	0
34th	0	0	0	0	0
35th	1 (2nd)	0	0	0	0
36th	0	0	0	0	0
37th	0	0	0	0	0
38th	0	0	0	0	0
39th	0	0	0	0	0
40th	0	0	0	0	0
41st	0	0	0	0	0
42nd	0	0	0	0	0
43rd	0	1 (1st)	0	0	0
44th	1 (3rd)	0	0	0	0
45th	0	0	0	0	0
46th	0	0	0	0	0
47th	0	0	0	0	0
48th	0	0	0	0	0
49th	0	0	0	0	0
50th	0	0	0	0	0
Total no. positive.....	7	23	2	4	2

* Figures in parentheses indicate larval stage.

labranchiae. When larvae were obtained, one larva was placed in a dipper of the sort described above and mechanically disturbed by poking at it. The time period during which the larva avoided the surface was then read from a stop-watch and recorded. In most cases 10 trials were made with each larva, after which it was discarded. The results of these trials are shown grouped by convenient frequencies in table 2. These

TABLE 2

Water surface avoidance by Sardinian Anopheline larvae following mechanical disturbance

LARVAE SUBMERGED IN EXCESS OF THE FOLLOWING TIME INTERVAL* MIN.:SEC.	*Anopheles l. labranchiae*		*Anopheles claviger*		*Anopheles hispaniola*		*Anopheles algeriensis*	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
:01	328	100	300	100	258	100	31	100
:02	175	53	281	94	255	99	29	94
:03	86	26	257	86	245	95	28	91
:04	44	13	243	81	243	94	28	91
:05	23	7	235	78	241	93	27	87
:10	16	5	209	70	225	87	26	84
:20	12	4	198	66	185	72	24	73
:30	9	3	184	61	147	58	21	68
:40	9	3	157	52	125	48	20	65
:50	6	2	134	45	104	40	16	52
1:00	6	2	116	39	93	36	14	45
2:00	5	2	52	17	54	21	6	19
3:00	2	1	25	8	36	14	3	10
4:00			17	6	25	10	1	3
5:00			12	4	19	7		
6:00			8	3	14	5		
7:00			7	2	11	4		
8:00			6	2	10	4		
9:00			5	2	9	3		
10:00			5	2	7	3		
11:00			5	2	3	1		
12:00			4	1	1	0.4		
13:00			2	0.7	1	0.4		
14:00			2	0.7	1	0.4		
35:00					1	0.4		

* Larvae which returned to the surface immediately are arbitrarily recorded under 1 second.

data show a marked difference between the behavior of *A. l. labranchiae* and the other three species studied. Thus after a lapse of five seconds all but seven per cent of the *A. l. labranchiae* had returned to the surface, while 78 to 93 per cent of the other species were still submerged, at 30 seconds the comparable figures are three per cent, contrasted with 58 to 68 per cent; at one minute two per cent contrasted with 36 to 45 per cent. At four minutes, in 328 trials, all the *labranchiae* larvae had returned to the surface but six per cent of the *claviger* and 10 per cent of the *A. hispaniola* were

still submerged. *Anopheles claviger*, in the course of 300 trials, was observed to remain below the surface for as long as 14 minutes, while in one of 258 trials with *hispaniola*, one larva remained submerged for as long as 35 minutes.

The ability of *Anopheles claviger* to remain submerged for long periods may be related to the fact that this species is apparently capable of cuticular respiration (Bates, 1949), passing the winter in the larval state, with the larvae capable of survival in water under ice. In Sardinia *claviger* and *hispaniola* larvae, which remained submerged the longest times in these tests, occur along streams in water which probably has a high dissolved oxygen content. This would aid the larvae in maintaining themselves by cuticular respiration under the water surface.

These data are significant in interpreting the adequacy of scouting for larvae by dipping at the surface, and possibly also in explaining the survival of larvae following treatment of water surfaces with oil-base larvicides which affect only the water surface.

On the average, scouts made 12 dips per man, per five minutes. Allowing five seconds for dumping the water from the dipper and making a fresh dip, the dipping interval would be about 20 seconds. According to the data given in table 2, 66 per cent of the *claviger*, 72 per cent of the *hispaniola*, and 73 per cent of the *algeriensis* would not have returned to the surface during this time period following the disturbance of the water surface. On the other hand, all but four per cent of the *labranchiae* would be at the surface after 20 seconds. When the larval densities are very low and the possibility of detecting them slight, even under the best conditions, a larger percentage of such larvae as might be present, of the species *claviger*, *hispaniola*, and *algeriensis*, would not be detected since they would not be at the surface.

The behavior of larvae in avoiding the water surface is also of importance in evaluating the degree of success that can be expected from the use of oil-base larvicides which are presumed to produce kills of larvae at the water surface. Concerned particularly are the stream-breeding species *claviger*, and *hispaniola*, which, as shown above, demonstrate significant surface avoidance following disturbance of the water surface. Oil-base larvicides fortified with toxicants such as DDT, and even with added spreading agents, may not be expected to persist on moving water for prolonged periods. Following the disturbance of the water by the movements of the larvicider, surface avoidance exhibited by *claviger* and *hispaniola* might well be expected to result in their failure to return to the water surface before the oil surface film was dissipated. This may in some measure explain why the ERLAAS experience showed a closer approach to eradication of *labranchiae* than *claviger* or *hispaniola* at the time when all ground water surfaces were being treated with larvicides although other factors may have been involved as well.

SUMMARY

Field experience in Sardinia illustrates how uncertain the method of dipping for anopheline larvae is in establishing their presence or absence, when populations are very low.

The premise that anopheline larvae are at or near the water surface is not always

valid. Experiments are reported demonstrating the extent of surface avoidance following mechanical disturbance for the species *Anopheles claviger*, *A. hispaniola*, *A. algeriensis*, and *A. l. labranchiae*. *Anopheles l. labranchiae* is shown to return to the surface more rapidly than the other three species. One minute after being disturbed, two per cent of the *labranchiae*, 39 per cent of the *claviger*, 36 per cent of the *hispaniola*, and 45 per cent of the *algeriensis* were still submerged. In 328 trials with *labranchiae* larvae the maximum submergence time was three minutes. The comparable figures for the other species were: *claviger*, 14 minutes in 300 trials; *hispaniola*, 35 minutes in 258 trials; *algeriensis*, 4 minutes in 31 trials.

REFERENCES

Bates, Marston 1941. Field studies of the anopheline mosquitoes of Albania. Proc. Ent. Soc. Wash., **43:** 37–58.

Bates, Marston 1949. The Natural History of Mosquitoes. MacMillan Co., New York, N. Y., 379 pp.

Logan, John 1950 *Anopheles labranchiae* eradication in Sardinia: An interim report. Amer. Jour. Trop. Med., **30:** 313–323.

Russell, Paul F., West, Luther S., and Manwell, Reginald D. 1946. Practical Malariology. W. B. Saunders Co., Philadelphia, Pa. 684 pp.

Soper, Fred L. and Wilson, D. Bruce 1943. *Anopheles gambiae* in Brazil, 1930 to 1940. The Rockefeller Foundation, New York, N. Y., 262 pp.

Shousha, Aly Tewfik 1948. Species eradication. The eradication of *Anopheles gambiae* from Upper Egypt, 1942–1945. Bull. World Health Org., **1:** 309–352.

RESUMEN

La experiencia adquirida en el campo en Sardinia demuestra cuán incierto el método de inmersión es para establecer la presencia o ausencia de larvas anofelinas cuando sus poblaciones son muy bajas.

La premisa que las larvas anofelinas se encuentran en o cerca de la superficie no es siempre válida. Se haa reportado experimentos que demuestran lo extensamente que las especies *Anopheles claviger*, *A. hispaniola*, *A. algeriensis y A. l. labranchiae* pueden evadir la superficie después de alterarse ésta mecánicamente. *A. l. labranchiae* parece retornar a la superficie más rápidamente que las otras tres especies. Un minuto después de la alteración dos por ciento de las *labranchiae*, 39 por ciento de *claviger*, 36 por ciento de *hispaniola* y 45 por ciento de *algeriensis* continuaron sumergidas. En 328 pruebas con larvas *labranchiae* el tiempo máximo de sumersión fué tres minutos. Las figuras comparables para las demás especies son: *claviger*, 14 minutos en 300 pruebas; *hispaniola*, 35 minutos en 258 pruebas; *algeriensis*, 4 minutos en 31 pruebas.

THE DURATION OF UNTREATED OR INADEQUATELY TREATED *PLASMODIUM FALCIPARUM* INFECTIONS IN THE HUMAN HOST

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During the past several years this laboratory has had occasion to treat a large number of neurosyphilitic patients with *Plasmodium falciparum*. The infection was studied in 22 patients, of an original group of 78, throughout its course to determine the total duration. An additional 16 patients were studied throughout the long primary period during which parasites were present continually in the peripheral blood. Our interest was due to the fact that literature on the subject of duration of infection is scanty and that a knowledge of the behavior of the parasite during the terminal stages of the infection might throw light upon such epidemiological problems as the persistence of this malaria in areas in which malaria transmission had been interrupted. The attrition of the group was due to the necessity of treating the malaria, deaths from neurosyphilis or other causes, and cure of neurosyphilis resulting in discharge of the patient.

So far as could be determined from the literature, no intensive study with regard to duration of infection has been made for this species of malaria. No such study was cited by Boyd (1949) in his comprehensive account of malaria, nor did he provide any specific information on the subject. Two studies on malaria therapy were found that did bear somewhat on the duration of *P. falciparum*. James, Nicol, and Shute (1932) observed the infection up to but not longer than six months after inoculation. Ciuca, *et al.* (1937) charted one patient with parasites up to 295 days after inoculation and other patients up to 100 or more days.

MATERIALS AND METHODS

The strain of malaria used in these experiments was brought from the field into this laboratory in June of 1946 from the vicinity of the Santee-Cooper reservoirs in the Coastal Plain of South Carolina. It has been designated as the Santee-Cooper strain of *P. falciparum* in this and other reports. All of the patients studied are, due to its recent establishment in the laboratory, close to the native infection in terms of number of passages.

The patients studied were neurosyphilitic Negroes, both male and female. They were inoculated either by means of sporozoites or by the intravenous injection of infected, citrated whole blood. The prepatent period varied from seven to 13 days in the mosquito-inoculated cases and from one to several days in the blood-inoculated cases. Blood smear follow-up was begun immediately in the latter cases and after five days in the former. Daily blood smears were taken (except sometimes on Sunday), until one month after the last parasites had been seen. After this, smears were taken

twice-weekly until six months had elapsed from the time parasites were last seen. In the event renewed parasite activity was observed, daily smears were reinitiated and the same protocol followed once more. Because of the frequent existence of a tertian parasite cycle, a patient was defined as free of parasites only if three consecutive smears failed to show organisms in the peripheral blood.

Blood smears were made by the direct Earle and Perez (1932) thick film method and 0.1 mm^3 of blood was routinely examined except in the case of high parasitemias when a smaller volume was sufficient. Giemsa stain was used for staining the smears and precautions were taken, by isolating slides expected to be without parasites, to prevent artifacts due to adherence of detached parasites washed off other smears.

Readings of the patients' temperatures were initiated at the same time as blood smears and were made every four hours during the clinical stages of the disease except during febrile periods when hourly or more frequent readings were made. Patients were examined daily or more often when necessary by a physician and were routinely given liver and iron preparations. In the case of unfavorable physical reaction by the patients or in cases of dangerously high parasitemia, the infection was terminated or kept at low level by means of antimalarial drugs. In the latter case the dosage was five grains of quinine sulfate, repeated if response was not satisfactory.

Due to the lack of uniformity in the definition of the term relapse, this designation has been avoided consciously in the pages which follow. We have attempted to describe the parasitemia pattern without the use of specialized terms.

OBSERVATIONS

Of 78 patients inoculated for the study, only 38 were followed for a sufficient length of time to be included in this report. Thirteen of these were inoculated with sporozoites and 25 with infected blood. The decrease in size of the group was due to several causes, by far the greatest being the treatment of malaria due to high parasitemia or unfavorable clinical reaction. Of the 38 patients remaining, 22, 9 with sporozoite-induced and 13 with blood-induced malaria, were followed throughout the duration of their infection and for six months after the last parasites were seen. The entire group of 38 was observed throughout the long primary period during which the patients had continuous parasitemia.

Before proceeding to a detailed treatment the general pattern of the *P. falciparum* infection will be outlined. After an incubation period corresponding closely with the prepatent periods previously mentioned, a clinical attack ensued varying from practically asymptomatic to severe. This attack was sometimes followed by one or more periods of renewed clinical activity. The clinical period was then followed by a period without symptoms during which the patients continuously had parasites in the peripheral blood. After the period of continuous parasitemia most patients had a period of varying duration during which parasites were intermittently present in the peripheral blood. The following paragraphs will describe these periods in turn.

Features of the clinical attack: Much information is available on the clinical characteristics of *P. falciparum* malaria; consequently, our observations were confined to those features which might be related to the overall duration of the disease.

Table 1 summarizes our observations. It was noted that there was only one clinical

episode in most of the cases observed. This indicates a strain of low virulence (see James *et al.* 1932, for a comparison of a mild and a virulent strain), as does the fact that about two-thirds of our original group of patients were able to terminate clinical symptoms spontaneously without the aid of drugs. In general the characteristics observed conform to those usually noted for this disease (Kitchen, 1949).

Period of continuous, remittent parasitemia: Following and including the one or more clinical attacks, malaria parasites were present continuously in the blood stream for a varying period. This period was characterized by waves of asexual parasites followed closely by waves of gametocytes producing the parasitemia patterns shown in figures 1 and 2. The height of parasitemia in the successive waves tended to become lower and lower as time elapsed. In most patients the successive peaks appeared to follow a cycle averaging in the neighborhood of three weeks, but due to irregularities accurate measurement of the cycle interval was not possible. Toward the end of the period densities of only a few parasites per mm^3 of blood prevailed.

TABLE 1

Characteristics of the clinical attack with the Santee-Cooper strain of Plasmodium falciparum*

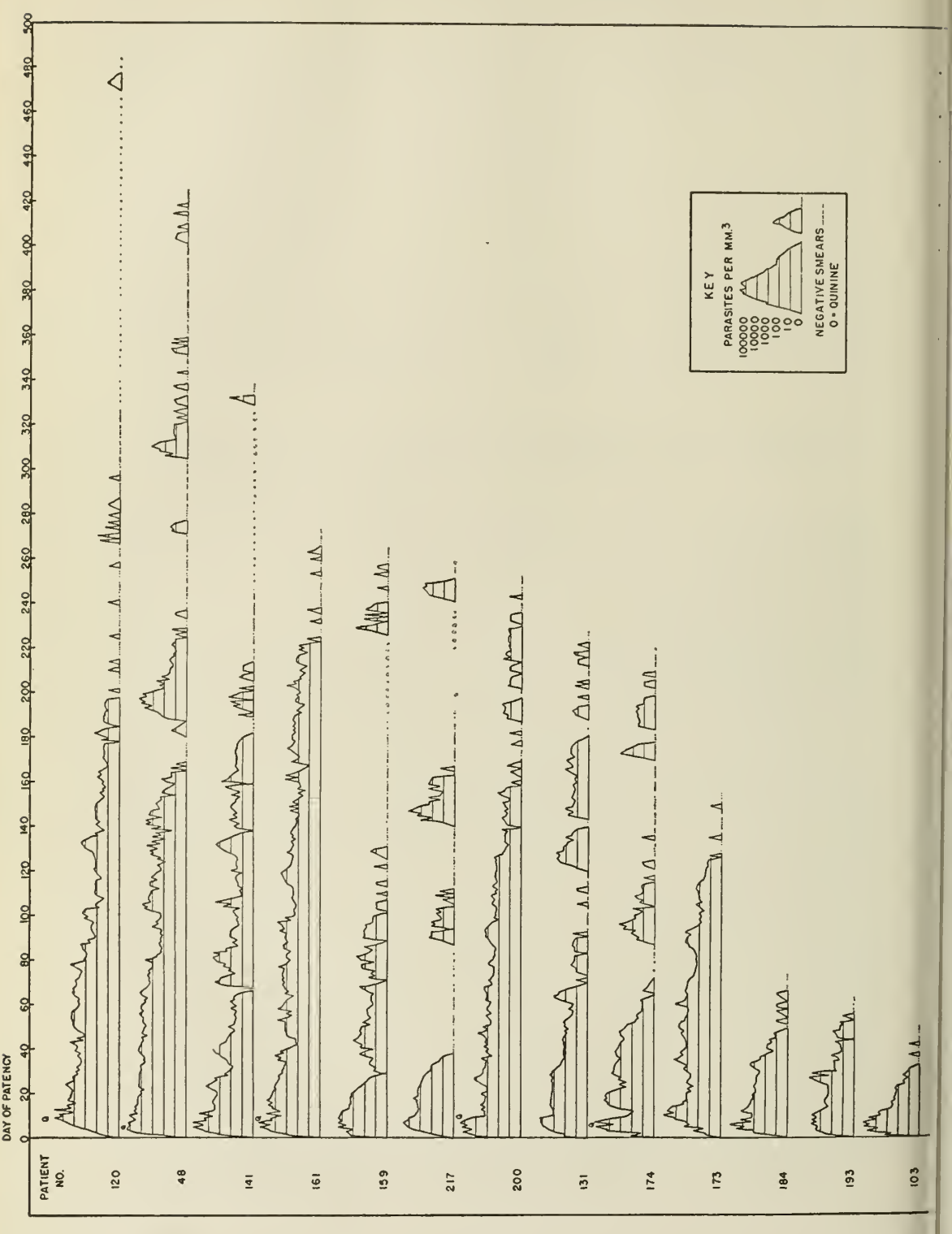
	SPOROZOITE INOCULATION	BLOOD INOCULATION	ALL
Number of patients	13	25	38
Number whose infection was interrupted with quinine	2	6	8
Mean length of initial clinical episode (days)	9.1	6.1	7.0
Mean number of clinical episodes	1.4	1.5	1.5
Mean total hours of fever over 101° F. oral	90.4	47.6	60.5
Median maximum parasite count (per mm^3)	65,000	79,000	74,000

* The clinical attack is defined as not only the first symptomatic period but symptomatic episodes associated with secondary waves of parasites occurring a short time after the initial episode.

The mean length of this initial period, as shown in table 2, during which parasites were always present in detectable numbers in the blood was 121 ± 9 days (median 120 days). The variation was great (standard deviation, 58 days), the extremes being 32 and 224 days after the beginning of patency. The blood and sporozoite induced infections appeared to behave similarly, the mean in the former being 113 days and the latter, 122 days.

Terminal period of intermittent parasitemia: Most patients (one exception among 38) after having had parasites in the blood continuously for a period up to several months continued to have parasites intermittently after this period (see figures 1 and 2). These episodes of parasitemia occurred in cycles similar to the peak cycles before the parasitemia became discontinuous. Again the successive peak densities of parasitemia were lower as time elapsed and the duration of each episode of parasitemia became more brief, until toward the last only occasional parasites were found. Reciprocally, the intervals during which parasites could not be detected became progressively longer. The length of this terminal period was similar to that of the initial continuous parasitemia period averaging 100 days (table 2).

Overall duration of the infection: In 22 cases, followed until no parasites had been


DAY OF PATENCY
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
PATIENT NO.
120
48
141
161
159
217
200
131
174
173
184
193
103
KEY
PARASITES PER MM.3
100000
10000
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NEGATIVE SMEARS
O = QUININE

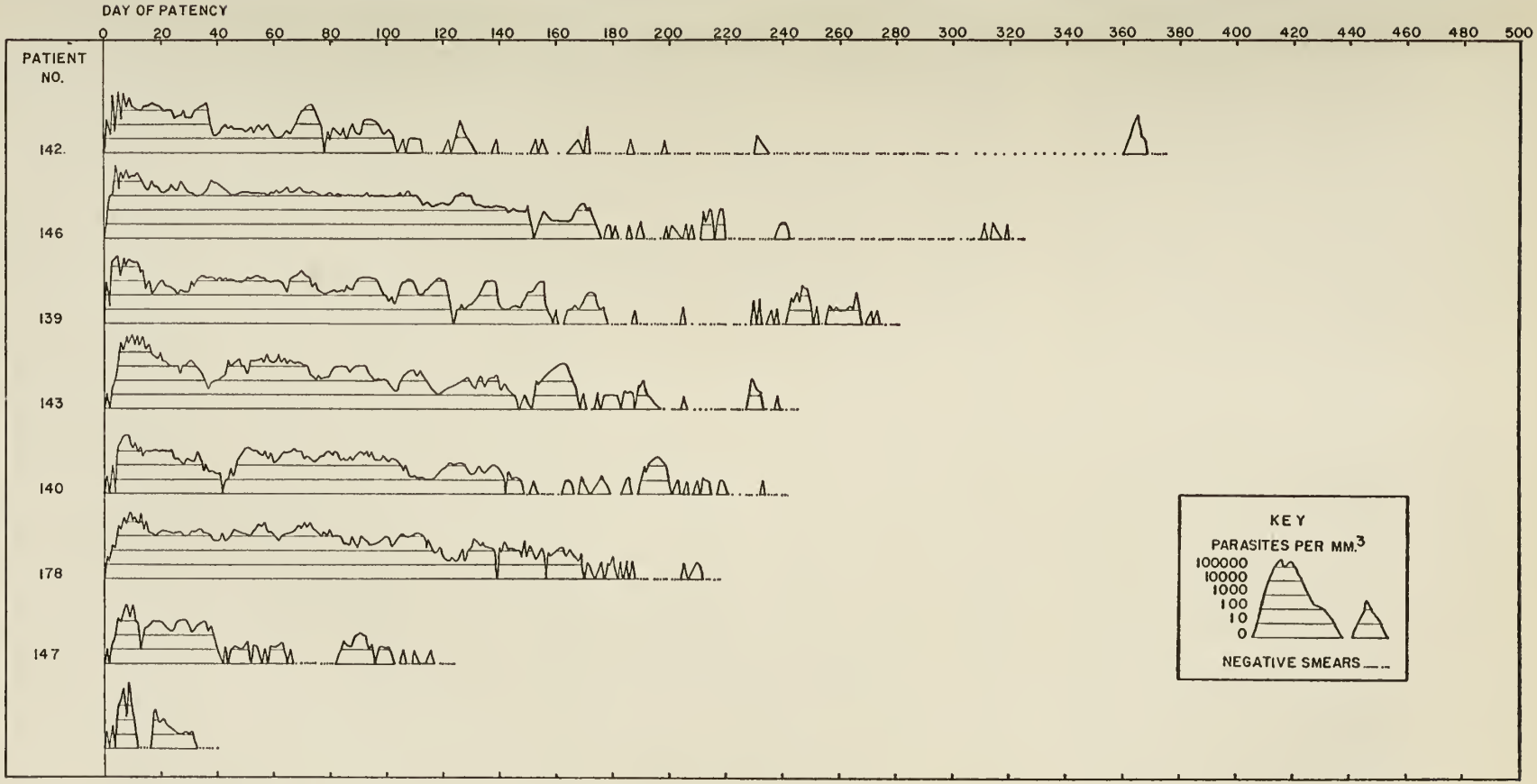

FIG. 2. Parasitemia of *Plasmodium falciparum* in untreated or inadequately treated patients. Sporozoite-induced infections. The number of patients figured is one less than the number studied. One sporozoite-induced infection was not graphed due to the presence of a concurrent *P. vivax* infection which made plotting impracticable.

seen for a period of six months, the mean total duration of the infection was 222 ± 25 days (median 236 days) (table 2). As described above, about half this period was constituted by the continuous parasitemia and the ensuing half by a period of intermittent parasitemia (figures 1 and 2). Variation in duration was considerable (standard deviation, 117 days) the extremes being 32 and 480 days. Duration in sporozoite- and blood-induced infections seemed similar.

In an effort to utilize the data as a basis for prediction, the distribution of overall duration was studied, and was found to fit a normal frequency distribution curve (chi-square, 4.89 with eight degrees of freedom) as shown in figure 3. Basing estimates upon this curve, it would be expected that five per cent of the cases would endure for 456 days or longer (the mean plus two standard deviations). We observed one in 22 cases (approximately five per cent) to last this long. Furthermore, one per cent of

TABLE 2

Summary of observations on the duration (in days) of the Plasmodium falciparum *infection*

	SPOROZOITE INOCULATION	BLOOD INOCULATION	ALL CASES
Total duration			
Mean and standard error	215 ± 35	226 ± 37	222 ± 25
Standard deviation	104	130	117
Extremes	32, 367	42, 480	32, 480
Duration of intial period of continuous parasitemia			
Mean and standard error	122 ± 16	113 ± 12	121 ± 9
Standard deviation	58	59	58
Extremes	32, 191	33, 224	32, 224
Duration of terminal, intermittent parasitemia			
Mean and standard error	87 ± 24	110 ± 26	100 ± 21
Standard deviation	73	93	85
Extremes	0, 254	0, 283	0, 283

the cases would be expected to endure for 492 days or longer (mean plus 2.6 standard deviations). These estimates will be considered further in the discussion.

Relation of duration of infection to other characteristics of the disease: To determine if other features of the disease might be related to the duration of infection the correlation coefficient was calculated for maximum height of parasitemia and overall duration. This was found to be positive and of moderate degree (r = +0.463*). Application of the *t* test proved this correlation to be of significance (p = between 0.05 and 0.02). This would indicate that the more severe parasitemias in the initial stages are likely to endure for a longer period of time than the less severe; which is to say, the mechanism of the human host which limits the degree to which the parasites can multiply in the early phases of the attack is related to, if not the same, as the mechanism which limits the length of time the parasites remain in the blood stream.

* Actually correlation was measured between the logarithm of the maximum parasite count, as the use of this transformation renders the frequency distribution of parasite number more nearly normal.

A similar correlation test was run between maximum parasite count and the length of the initial period of continuous parasitemia. In this case the correlation was also also positive and greater in degree (r = +0.531). Test of significance produced a less equivocal result than in the previous case (p = less than .001). This finding would be subject to the same interpretation as the preceding.

Finally, the degree of correlation was measured between the length of the continuous parasitemia period and the length of the intermittent parasitemia period which followed. The correlation was positive but low and insignificant (r = +.244;

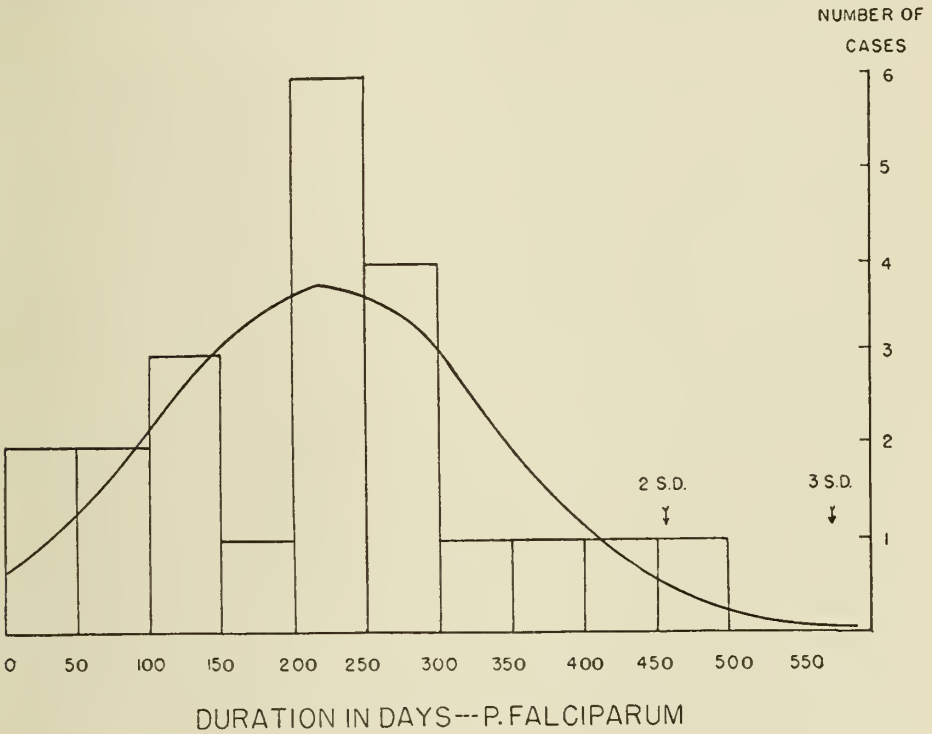

FIG. 3. Duration of *Plasmodium falciparum* infection fitted to a normal frequency distribution curve.

p = .30). Upon the basis of this, it would appear that the duration of the intermittent parasitemia period may be independent of the duration of the continuous parasitemia, or if the two are related then the relationship must be slight.

DISCUSSION

With the reservation that laboratory findings must be applied to field conditions with caution, the observations reported in this paper can be used in explaining some puzzling field findings.

For instance, the existence of a long period of intermittent parasitemia provides a logical explanation for the finding of parasites in the blood of persons in malaria control areas who had previously been found parasite free on one or even several

examinations. As an example from our data, patient number 48 (figure 1) had parasitemia intermittently from the 170th through the 418th day. Over this 248 day period the patient was examined 209 times during which time parasites were present on 76 days and absent on 133. Thus there would only be about one chance in three, on the average, of finding this patient with parasites during the intermittent parasitemia period. Examination of the figures will show that this patient is not exceptional although much variation is present from patient to patient.

Similarly, the data presented on overall duration of infection might be used in estimating how long observation must be continued to demonstrate the effectiveness of malaria control. It was stated earlier, on the basis of the curve in figure 3, that five per cent of the cases would be expected to endure 456 days or more. On the same basis 22 per cent would be expected to endure a year or be positive the following malaria transmission season (i.e. May through November in the Middle South). If infections occurred late in the autumn about one case in 100 would be expected to have parasites during two succeeding malaria transmission seasons; however, the chances of an infection persisting into a third malaria season after that in which infection occurred would be extremely small (less than 0.0000001).

Considerations which might modify these deductions are as follows: 1. the possibility that strains of *P. falciparum* from other localities might differ as to duration; 2. the possibility that our assumption of normality for the distribution of duration is in error; 3. the fact that severe attacks in our patients were treated, resulting in the selection of a group which was not representative as regards intensity of infection; 4. the fact that some of our patients received anti-luetic therapy (penicillin, mapharsen, and tryparsamide) during the course of our observations.

With regard to the first, we are now studying a strain of *P. falciparum* from Panama; no notable differences have been observed. As to the second, a great deviation from normality would be necessary to significantly modify our conclusion as to persistence into a third malaria season. The fact that we terminated severe infections biases our estimate of duration toward the low side since height of parasitemia and duration were positively correlated. In this regard, however, it must be remembered that in nature many of the severe infections would result in death, thus providing a selection similar to that made by us. Finally, we do not believe that the anti-luetic therapy influenced the parasitemia to any material degree.

Despite these limitations one might predict that, in the presumed absence of transmission, parasite carriers detected through the second malaria season after the last season during which transmission occurred freely could represent old infections. However, if parasite carriers were found after the second transmission-free season, one might investigate to determine if transmission had been resumed.

The results of our study on the persistence of the *P. falciparum* infection confirm deductions from field observations as to the means by which this parasite survives the winter in temperate regions. Since over half of the infections persisted for seven and one-half months it would be expected that half of the cases occurring in mid-November would still be patent, at least intermittently, at the end of the following May. In the more southern part of the United States these two hypothetical dates fall in seasons in which the vector is active. It was also pointed out earlier that 22 per cent of the cases would be patent intermittently at the end of a year, so a substan-

tial portion of the persons infected during the peak of transmission during one year would provide a potential source of infection for the vector during the same period of the following year. In a separate study, now in preparation for publication, we are investigating the infectivity of these terminal parasitemias to mosquitoes. Suffice it to say that infections were frequently secured despite the extremely low parasite counts. In this regard, Young *et al.* (1948) found that some field subjects with low parasite densities in the Santee-Cooper region were infective to mosquitoes. Many of these parasitemias must have been of the same type as the terminal parasitemias described in this study.

SUMMARY AND CONCLUSIONS

A study of 22 patients with *Plasmodium falciparum* infections showed that the parasitemias persisted for an average of 222 ± 25 days. Three of the parasitemias persisted for more than one year, the longest being 480 days. A study of the frequency distribution of total duration fitted to a normal curve led to the prediction that about one per cent of the infections, if contracted late during one malaria transmission season, might persist through the following and into the second following malaria season. Assuming a two year period during which transmission had not occurred, infections found subsequently would not be the result of a persistent infection but would be due to transmission from an introduced carrier.

A study of 38 patients, including the previous 22, showed that the infection was initiated by a continuous parasitemia which endured on the average 121 ± 9 days, the clinical portion of the infection being included in the first few days of this period. During the continuous parasitemia period there were cyclic increases and decreases in parasite number but successive cycle peaks tended to be progressively lower until densities of only a few parasites per mm^3 prevailed.

Following the continuous phase, parasitemia was of an intermittent, cyclic type with each successive peak generally attaining a lower parasite density. This period averaged 100 ± 21 days.

It was concluded that the intermittent nature of the terminal parasitemia would explain the finding of parasites in persons who had previously been found free of parasites for one or more examinations without necessitating the postulation of renewed malaria transmission. The long duration observed confirms the field deduction that *P. falciparum* passes the winter in temperate zones in the human host.

It was found that total duration was positively correlated with maximum parasite count during the clinical phase of the infection, as was the duration of the period of continuous parasitemia. Duration of intermittent parasitemia could not be shown to bear any relationship to the duration of the continuous parasitemia which preceded it. From this it was concluded that related factors (if not the same factor) are involved in the mechanism which limits the height to which the parasites multiply during the initial stages of the parasitemia and the mechanism which determines how long parasites will persist in the human host.

REFERENCES

Boyd, M. F. 1949. Malariology. 2 vols. W. B. Saunders Company, Philadelphia.

Ciuca, M., Ballif, L., Chelarescu, M. and Lavrinenko, N. 1937. IV. Contribution a l'etude

del'infection experimentale au Pl. falciparum. Sonderabruck aus der Festschrift Nockt 1937, Inst. f. Schiffe-u. Tropenkrankheiten, Hamburg. pp. 81–101.

Earle, W. C. and Perez, M. 1932. Enumeration of parasites in the blood of malarial patients. Jour. Lab. and Clin. Med., **17**: 1124–1130.

James, S. P., Nicol, W. D. and Shute, P. G. 1932. A study of induced malignant tertian malaria. Proc. Roy. Soc. Med., Section of Trop. Dis. and Parasit., **25**: 1153–1186.

Kitchen, S. F. 1949. *Falciparum* malaria. In Boyd, M. F. Malariology, II: 995–1016. W. B. Saunders Company, Philadelphia.

Young, M. D., Hardman, N. F., Burgess, R. W., Frohne, W. C., and Sabrosky, C. W. 1948. The infectivity of native malarias in South Carolina to *Anopheles quadrimaculatus*. Amer. Jour. Trop. Med. **28**: 303–311.

RESUMEN Y CONCLUSIONES

El estudio de 22 pacientes infectados con *Plasmodium falciparum* demostró que las parasitemias persistieron durante un período medio de 222 ± 25 días. Tres de las parasitemias persistieron durante más de un año, la más larga extendiéndose a 480 días. El estudio de la distribución de frecuencia en la duración total aplicada a una curva normal nos permitió predecir que aproximadamente un por ciento de las infecciones contraídas a fines de la estación de transmisión de malaria podrían persistir durante la próxima o durante la subsiguiente estación malárca. Si asumimos un período de dos años libre de transmisión entonces la infecciones subsiguientes no serían debido a una infección persistente sino que a la transmisión por medio de un nuevo portador de la enfermedad.

Un estudio en 38 pacientes, incluyendo los 22 anteriores, demostró que la infección fué iniciada por una parasitemia continua que duró 121 ± 9 días, la fase clínica de la infección siendo incluída durante los primeros días de este período. Durante el período de parasitemia continua se registraron aumentos y descensos cíclicos en el número de parásitos pero los valores máximos de los ciclos sucesivos decrecieron progresivamente hasta que prevalecieron densidades de solamente pocos parásitos por milímetro cúbico.

Después de su período continuo la parasitemia se volvió intermitente y cada valor máximo sucesivo generalmente demostró menor densidad parasítica. Este período alcanzó un promedio de 100 ± 21 días.

Se concluyó que la naturaleza intermitente de la parasitemia terminal podría explicar el hallazgo de parásitos en personas que anteriormente se habían declarado negativas después de uno a más exámenes sin tener que postular acerca de una nueva transmisión de malaria. La larga duración observada confirma la deducción que *Plasmodium falciparum* inverna en el huésped humano de las zonas templadas.

Se observó una correlación positiva entre la duración total y el período de parasitemia continua y el contaje máximo de parásitos durante el período clínico de la infección. La duración de la parasitemia intermitente no se relacionó con el período de parasitemia continua que la precedió. Se concluye por lo tanto que factores relacionados (o el mismo factor) toman parte en el mecanismo que limita la intensidad de reproducción de los parásitos durante los estados iniciales de la parasitemia y el mecanismo que determina cuanto tiempo los parásitos persistirán en el huésped humano.

OBSERVATIONS ON A GAMETOCYTELESS STRAIN OF *PLASMODIUM FALCIPARUM*

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Very little information has been published concerning strains of *Plasmodium* which have lost the ability to complete the sporogonous cycle in the intermediate host. On a number of isolated occasions reports have appeared in the literature concerning the loss of gametocytes in human malaria, but very often it has been shown that this loss was only transitory, or that the gametocytes were reduced somewhat in numbers and the investigators overlooked the existing forms, or failed to distinguish them from the asexual forms.

Perhaps the most complete information concerning the loss of gametocytes in *Plasmodium falciparum* is in the work of Boyd (1945). In reporting a number of factors influencing the experimental propagation of *P. falciparum*, Boyd noted that during artificial propagation in certain strains of this parasite, gametogeny deteriorated either suddenly or gradually, the gametocytes appearing only in very small numbers, or even disappearing completely. When these changes appeared they persisted throughout subsequent passages. Boyd also reported that one strain (Trinidad) was completely agametocytogenic during its last nine blood passages. This strain apparently became gametocyteless after 16 blood passages.

The present work reports the finding of a similar gametocyteless strain of *P. falciparum*. The original strain was one designated as P. f. ✻5 (Eyles and Young, 1950) and was recovered from a Negro living in the vicinity of the Santee-Cooper impoundments in South Carolina during the summer of 1946.

OBSERVATIONS

Following isolation of the parent strain by blood inoculation, seven lineal blood transfers were made. At this point the infection was transmitted by mosquitoes to patient number G 178 (figure 1), and this was followed by seven further lineal blood inoculations. Two patients (G 237 and G 240) were inoculated from a single source in this seventh lineal transfer in early October, 1948. Late in that same month it became apparent that blood smears taken regularly from these two patients at that time failed to show any gametocytes, when normal examination procedures were used. Subinoculations were then made from both of these patients. From one (G 237) a total of 19 lineal blood passages have been made, as well as several collateral transfers. From the second (G 240) a single lineal transfer was made. Table 1 (B) summarizes the data available on these patients, and figure 1 indicates the lineage of the strain, using the last mosquito-inoculated case as a starting point. It will be noted that the apparent loss of gametocytes occurred after the seventh blood passage.

With two exceptions, no gametocytes have been noted in any of these patients on

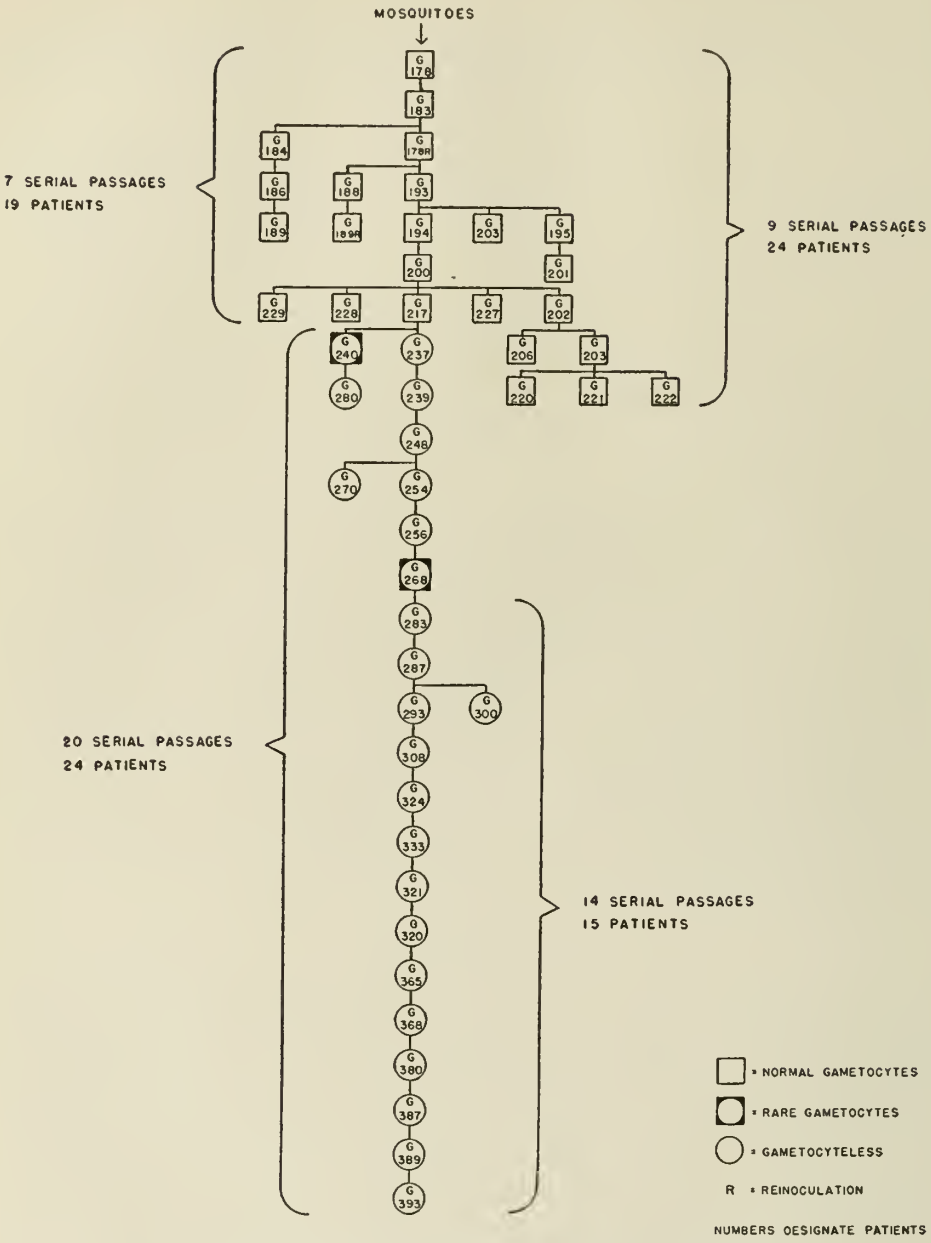

FIG. 1. History of the gametocyteless strain of *Plasmodium falciparum*

any of the days examined, although blood smears of the Earle-Pérez type (1932) were usually made daily, or at least on five days a week, for a total observation period of 4,651 patient-days. At least 0.1 cu.mm. of blood was examined from each blood smear. The exceptions to the absence of gametocytes occurred in two patients. The first was

TABLE 1

Parasitological observation on 36 P. falciparum *infections, comparing the periods before and after loss of gametocytes*

PATIENT NO.	NO. OF DAYS OBSERVED	LAST DAY OF PARASITES	NO. OF DAILY SMEARS	TOTAL POSITIVE DAYS	DAYS POSITIVE ASEXUAL PARASITES	DAYS POSITIVE GAMETOCYTES	MOSQUITO FEEDINGS	POSITIVE FEEDINGS
A. Characteristics of Infections preceding loss of gametocytes								
G 200	481	246	278	164	132	138	0	—
G 159	449	261	339	96	73	63	57	37
G 217	431	252	209	63	54	18	0	—
G 178	403	213	239	147	135	115	10	2
G 106	402	329	347	145	120	97	77	51
G 193	328	59	154	43	26	33	0	—
G 194	293	293	225	105	62	60	13	13
G 203	217	217	181	119	98	83	0	—
G 228	107	107	92	61	56	21	0	—
G 227	83	74	74	59	32	37	0	—
G 188	42	42	37	36	36	27	0	—
G 195	33	33	30	28	19	18	0	—
Total 12.....	3,269	215[1]	2,205	1,066	843	710	157	103
B. Characteristics of Infections after loss of gametocytes								
G 240	467	244	289	88	87	1	1	0
G 280	248	231	209	152	152	0	5	0
G 237	317	313	226	106	106	0	1	0
G 239[2]	24	24	21	17	17	0	0	—
G 248	176	158	127	76	76	0	1	0
G 254	52	31	33	17	17	0	0	—
G 270	79	72	52	46	46	0	0	—
G 256	282	277	225	171	171	0	7	0
G 268	444	376	350	80	79	1	10	0
G 283	48	45	44	34	34	0	2	0
G 287	144	141	118	113	113	0	1	0
G 293	254	166	193	125	125	0	6	0
G 300	81	78	71	58	58	0	2	0
G 308	263	138	178	116	116	0	1	0
G 324	201	102	153	72	72	0	4	0
G 333	86	83	75	60	60	0	0	—
G 321	207	189	186	150	150	0	0	—
G 320	165	130	135	76	76	0	0	—
G 365[2]	15	14	15	11	11	0	0	—
G 368	324	284	159	37	37	0	2	0
G 380	115	112	101	98	98	0	3	0
G 387	299	199	197	131	131	0	6	0
G 389	120	120	101	97	97	0	0	—
G 393	240	234	181	128	128	0	0	—
Total 24.....	4,651	139.5[1]	3,439	2,059	2,057	2	52	0

[1] Median.

[2] Patient terminated too early for adequate observation.

a single gametocyte found in an entire thick smear, or 0.5 cu.mm. of blood, taken from patient G 240 (7th lineal blood passage, figure 1) after 212 days of observation of this patient. The other was in the 12th lineal passage (figure 1), when two gametocytes were found after careful search of an entire thick smear (0.5 cu.mm. of blood) from patient G 268 after 57 days of observation. A total of 3,439 thick films have been examined from the 24 gametocyteless patients, of which 2,057 were positive for asexual parasites. The strain has been completely agametocytogenic during the last 14 lineal passages, as well as in seven of nine patients observed during the six earlier lineal transfers.

For the sake of comparison table 1 (A) is included. The patients included in this table are those in the same line of transfer previous to the gametocyteless patients, as well as several other patients from collateral lines of parasite transfer. This table illustrates the frequency of appearance of gametocytes in the parent strain before it became gametocyteless.

Mosquito feedings (*Anopheles quadrimaculatus*) have been made on patients with these infections on 52 different occasions, including the two days on which gametocytes were noted (table 1 B). Twenty-five to 50, with an average of 36, specimens were dissected from each lot of mosquitoes fed, and no infected specimens were found. By comparison, 103 of 157 lots of mosquitoes fed on four patients carrying the parent strain, while gametocytes were present, became infected (table 1A).

DISCUSSION

A number of factors have been considered in attempting to explain the loss of gametocytes in this strain. Gametocytes disappeared to all practical purposes after the parent strain had been subjected to seven lineal blood passages, but it would seem that the mechanism of loss of gametocyte production is more complicated than just the result of a certain number of artificial passages. The parent strain originally was subjected to eight linear blood transfers before a mosquito passage intervened, and seven lineal blood passages later gametocytes disappeared in one branch of the strain. In a closely associated collateral branch of the parent strain, beginning with patient G 202 (figure 1), involving six additional patients and two additional lineal passages, gametocytes did not disappear. It is obvious that gametocytes did not disappear simultaneously in all branches of the strain. In an earlier collateral line the parasite had been subjected to as many as 11 serial blood passages without loss of gametocytes.

According to our observations (table 2) there was little noticeable drop in maximum gametocyte densities from early blood passages to later ones. There was a considerable amount of variation in these densities from patient to patient, as might be expected from differences in patient immunity, or possibly because of the administration of small doses of quinine to suppress clinical manifestations in some cases. The sudden disappearance of the gametocytes followed transmission from a donor in which there was a maximum gametocyte number of 6,600 per cu.mm., a moderately high density. In three other patients collateral with this donor the maximum gametocyte densities were 2,268, 1,680, and 1,290 per cu.mm., respectively. Subinoculations from one of these (G 202, with 2,268 gametocytes per cu.mm.) resulted in normal gametocyte densities in the recipients.

One possible explanation for loss of gametocytes might be the extraordinary long period of low subclinical parasitemia in the donor, prior to the inoculation of the two original gametocyteless patients (G 237 and G 240). Blood inoculations are usually done 10 to 30 days after inoculation of the donor, i.e. during the primary parasitemia. In patients G 237 and G 240, 161 and 162 days had elapsed since the inoculation of the donor, and these subinoculations were done during the second appearance of asexual parasites, after the spontaneous termination of the primary attack. No earlier subinoculations had been made from this donor, so it is impossible to determine any direct differences in the ensuing infections. However, several other similar cases have been studied, to determine any differences in infections derived from early and late subinoculations.

Table 3 shows the gametocyte densities in a number of recipients who received blood inoculations from four donors at varying intervals after infection was intro-

TABLE 2

Gametocyte densities in eleven artificial blood passages preceding and parallel to the gametocyteless line

PASSAGE	NUMBER OF PATIENTS	MAXIMUM GAMETOCYTES PER CU. MM.		
		Range		Average
		From	To	
1	2	380	3,040	1,710
2	5	230	10,160	4,905
3	3	410	9,040	5,003
4	6	360	36,480	10,199
5	4	1,350	7,704	3,911
6	12	620	7,500	3,592
7	9	2,680	18,180	6,384
8	6	520	4,240	2,702
9	1	1,500	1,500	1,500
10	2	1,660	2,140	1,900
11	1	6,060	6,060	6,060

duced in the donors. These recipients were subinoculated from 10 to 175 days following inoculation of the donors; there was no evidence of consistent differences in maximum gametocyte densities in these patients, or in the ratios of gametocyte frequency to frequency of asexual stages, in relation to stage of infection in the donor. Table 4 summarizes the average maximum gametocyte densities in three groups of 12 patients, each group inoculated from donors who had carried the infection for short, medium and long periods of time before subinoculation. Little difference is apparent in the average maximum gametocyte densities, although there is a wide range in all three groups.

Comparing the gametocyte productivity of the two methods of inoculation, there does not seem to be any superiority in mosquito transmission over blood transmission. Table 5 summarizes the results of 13 mosquito inoculations; the mosquitoes acquired their infection from two blood-inoculated donors. The average maximum gametocyte densities in these recipients were considerably lower than those found in the blood-

TABLE 3

Gametocyte densities in patients' blood inoculated from donors in various stages of infection

DONORS						RECIPIENTS				
Donor No.	Maximum gametocytes per cu. mm.	Days asexual forms present	Days gametocytes present	Ratio of days gametocytes to asexual forms	Day of inoculation to recipient	Recipient No.	Maximum gametocytes per cu. mm.	Days asexual forms present	Days gametocytes present	Ratio of days gametocytes to asexual forms
G-200	1,350	132	138	1.05	10	G-202	2,268	13	12	0.92
					66	G-217	6,600	49	11	0.37
					155	G-227	1,680	32	37	1.16
					155	G-228	1,290	56	21	0.38
G-120	4,848	140	154	1.10	61	G-131	2,760	65	104	1.60
					104	G-157	520	17	43	2.53
G-106	4,410	119	100	0.84	57	G-114	4,320	18	14	0.78
					57	G-115	2,360	54	43	0.80
					57	G-116	620	29	29	1.00
					61	G-117	3,510	79	73	0.92
					63	G-118	6,780	27	22	0.82
					63	G-119	7,500	27	23	0.85
					170	G-156	4,520	76	49	0.65
G- 48	3,040	195	160	0.82	24	G- 54	2,540	20	21	1.05
					175	G- 93	2,860	32	32	1.00
					175	G- 94	10,160	22	26	1.18
Averages.....	3,412	146.5	138.0	0.94			3,768	38.5	35.4	0.92

TABLE 4

Summary of gametocyte densities in 36 patients inoculated from donors on different days of patency

DAYS OF PARASITE PATENCY IN DONOR		RECIPIENTS			
Range of days	Number of Average days	Number of cases	Maximum number of gametocytes per cu. mm.		
			Range		Average
			From	To	
6– 10	8.0	12	1,664	8,736	4,768
11– 25	17.8	12	230	18,180	4,803
29–170	104.8	12	1,290	10,160	4,466

inoculated cases (cf. tables 2 and 3 for average maximum gametocyte densities in blood-inoculated cases). The ratio of gametocyte frequency to frequency of asexual parasites conforms very closely to that found in blood inoculations. (cf. table 3).

The gametocyteless strain has lost none of its qualities for producing high asexual parasitemias in the patient, or in producing good clinical cases of malaria. Another strain characteristic which is apparently unaltered is the persistence and recurrence

of asymptomatic parasitemias for long periods of time. In work on the parent strain, Eyles and Young (1951) observed parasites for as long as 367 days after patency following sporozoite inoculation, and for 480 days after blood inoculation; the mean total duration for all cases was 222 days. During the current study similar prolonged asymptomatic parasitemias were noted. The mean duration in 14 uninterrupted cases was 217 days.

The parent strain is known to be quite infective to *A. quadrimaculatus* (Eyles and Young, 1951). It was possible to infect mosquitoes on patients as late as 321 days after the primary attack; it was found also that a very low gametocyte threshold sufficed to infect mosquitoes. Several patients had parasitemias sufficient to infect

TABLE 5

Occurrence of gametocytes in blood-inoculated donors as compared to mosquito-inoculated recipients

BLOOD-INOCULATED DONORS					MOSQUITO-INOCULATED RECIPIENTS				
Patient no.	Maximum gametocytes per cu. mm.	Days asexual forms present	Days gametocytes present	Ratio of days gametocytes to asexual forms	Patient no.	Maximum gametocytes per cu. mm.	Days asexual forms present	Days gametocytes present	Ratio of days gametocytes to asexual forms
G-120	4,848	140	154	1.10	G-132	3,260	81	99	1.22
					G-140	1,340	126	80	0.64
					G-139	450	149	107	0.72
					G-142	2,200	57	84	1.47
					G-143	240	124	106	0.86
					G-145	2,140	22	26	1.18
					G-146	4,880	132	138	1.05
					G-147	1,220	52	28	0.54
					G- 6	3,420	126	130	1.03
					G-105	1,664	49	31	0.63
					G-152	900	52	78	1.50
G-159	2,760	69	56	0.81	G-178	1,880	26	16	0.62
					G-179	520	40	49	1.23
Averages.....	3,804	104.5	105.0	1.01		1,855	79.7	74.8	0.94

mosquitoes when no gametocytes could be demonstrated by normal examination procedures, i.e., less than 10 gametocytes per cu.mm. The mosquito-feedings on the gametocyteless patients were usually done at a time when, according to observations on the parent strain, the gametocyte densities might be expected to be high. No infections were found in the 52 lots of mosquitoes fed.

Comparing these results with the success achieved by Eyles and Young in the infection of mosquitoes fed on subjects with very low gametocyte densities, it would seem fairly certain that infective gametocytes were not present even in sub-microscopic densities in the present cases. Even the rare sexual forms observed in the blood on a single occasion in two patients apparently were incapable of producing infection in the mosquito.

SUMMARY

A strain of *Plasmodium falciparum* was observed to become practically agametocytogenic after seven lineal artificial blood-passages. The strain was observed subsequently through 19 lineal passages, involving 24 patients, for a total observation period of 4,651 patient-days. Rare gametocytes were found on only two blood films during the whole period of observation. The strain was completely agametocytogenic during the last 14 lineal passages, as well as in seven of nine patients observed during the six earlier lineal transfers.

No infections were obtained in 1,865 mosquitoes in 52 lots, fed on the patients after the first loss of gametocytes.

The character of the parasitemias and of the clinical responses was similar, before and after the loss of gametocytes.

It is concluded that loss of gametocytes was not due simply to the number of artificial passages of the parasite. Other possible reasons were investigated, but the results seem to rule out any obvious explanation.

REFERENCES

Boyd, M. F. 1945. On difficulties arising in the experimental propagation of *falciparum* malaria. Amer. Jour. Trop. Med. **25**: 293–306.

Earle, W. C., and Pérez, M. 1932. Enumeration of parasites in the blood of malarial patients. Jour. Lab. and Clin. Med., **17**: 1124.

Eyles, D. E. and Young, M. D. 1950. The comparative susceptibility of *Anopheles albimanus* and *Anopheles quadrimaculatus* to a South Carolina strain of *Plasmodium falciparum*. Jour. Infect. Dis., **87**: 189–193.

Eyles, D. E. and Young, M. D. 1951. The duration of untreated or inadequately treated *Plasmodium falciparum* infections in the human host. Jour. Nat. Mal. Soc., **10**: 327–336.

RESUMEN

Se observó que una cepa de *Plasmodium falciparum* se convirtió en una casi enteramente libre de gametocitos después de siete pases lineares de sangre artificiales. Esta cepa se observó subsiguientemente a través de 19 pases lineares incluyendo 24 pacientes durante un período de observación total de 4,651 paciente-días. Observáronse muy pocos gametocitos en solamente dos laminillas de sangre durante todo el período de observación. La cepa demostró ser completamente no gametocitogénica durante los últimos catorce pases, así como en siete de los nueve pacientes observados durante los seis pases lineares anteriores.

No se obtuvieron infecciones en 1865 mosquitos distribuídos en 52 lotes alimentados en pacientes después de la primera pérdida de gametocitos.

La naturaleza de la parasitemia y de los resultados clínicos resultaron similares antes y después de la pérdida de gametocitos.

Se ha concluído que la pérdida de gametocitos no se debió simplemente al número de pases artificiales del parásito. Los resultados obtenidos en la investigación de ostras razones posibles parecen excluir una explicación simple.

PROMISING DDT-SYNERGIST COMBINATIONS FOR THE CONTROL OF RESISTANT FLIES

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During the past five years, the development of a high degree of resistance to DDT by field strains of the house fly, *Musca domestica*, Missiroli (1947), Wiesmann (1947), King and Gahan (1949), Bruce (1949), March and Metcalf (1949) and by field strains of certain species of mosquitoes (Deonier, et al., 1950) has posed important new problems in operational control programs against insect vectors of certain diseases. Various approaches have been made to the solution of the problem. One has been the use of other chlorinated hydrocarbon insecticides as residuals against the DDT-resistant house flies. This approach has been unsatisfactory for the most part since the insects were either initially resistant to the new insecticides (Knipling, 1950) or very rapidly developed resistance (March and Metcalf, 1949a) (Mosna, 1951). Combined deposits of the insecticides produced combined resistance (Pratt and Babers, 1950).

Several laboratories over the country have, therefore, followed a more basic approach aimed at determining the mode of action of DDT and related insecticides and the resistance-mechanism in the flies. It was discovered that DDT-resistant house flies were able to decompose DDT to form less toxic derivatives by physiological processes within their bodies (Sternberg, et al., 1950). Combinations of piperonyl cyclonene and DDT were found to increase the toxicity of the deposits against DDT-resistant flies, although no difference was evident against normal flies (Perry and Hoskins, 1950). Work with combinations of DDT and DMC [1,1-bis(*p*-chlorophenyl)-ethanol] demonstrated marked synergistic action, although the DMC by itself was relatively nontoxic to house flies (Sumerford, et al., 1951). It is possible that the individual chemicals added to the DDT deposits blocked the detoxification mechanism of the DDT-resistant flies and thus manifested a synergistic action. Extensive, rapid screening tests were made, therefore, with a view toward discovering other chemicals showing a synergistic action regardless of their toxicity to insects when applied alone.

TECHNIQUE

In screening compounds, a general formulation was used in which 0.5 per cent of the test chemical and 5 per cent DDT were combined in a methyl ethyl ketone solution. Each test solution was pipetted onto three poster-board test panels at the rate of 200 mg. of DDT and 20 mg. of test chemical per sq. ft.; then the residues were allowed to age for one week before testing. A field strain of house flies, found highly resistant to DDT and other halogenated hydrocarbon insecticides, was used in the evaluations. Approximately fifty 3-day-old adult flies were confined in a petri dish cage on the residual deposits for a 2-hour test period. The 24-hour mortal-

ities were taken as the criteria of effectiveness. Daily comparisons were made with residual deposits of 200 mg. of DDT per sq. ft. and also, with a combination of 200 mg. of DDT and 20 mg. of DMC per sq. ft. Those chemicals showing activity comparable to that of the DDT-DMC combination were retested using proportions of DDT to test chemical of 10:1, 5:1, and 1:1 based on an application of 200 mg. of DDT per sq. ft. The best synergistic combinations were evaluated further in stand-

TABLE 1

Twenty-four-hour mortalities of adult female M. dŏmestica *of a DDT-resistant strain after 2-hour exposures to 1-week-old residual deposits of 200 mg. of DDT and 20 mg. of test compound per sq.ft.*

Comparative mortalities from a deposit of 200 mg. of DDT and 20 mg. of DMC per sq. ft. are given. Mortalities from deposits of 200 mg. of DDT per sq. ft. were negligible in all cases.

TEST COMPOUND	24-HR. MORTALITY (PER CENT)	
	Test compound plus DDT	DMC plus DDT
Dihydroxyanthraquinone (a)	92	94
Quinhydrone	85	89
p-Chlorophenyl 1,2-dichloro-2-(*p*-chlorophenyl)-ethyl ketone (b)	92	88
2,4-Dichlorophenoxyfumaric acid	81	85
Isopropyl β-naphthoxyacetate	89	79
Di-*p*-nitrobenzyl terephthalate (c)	89	79
1,1-bis(*p*-Fluorophenyl)-ethanol	83	78
1,1-bis(*p*-Chlorophenyl)-ethane	73	78
Hydroxy-pentamethyl flavan	69	69
l-Xylose	65	66
p-Chlorobenzaldehyde semicarbazone	71	64
Phenylmercuric salt of 2,4-pentanedione	84	63
3-Hydroxy-2-naphthoic acid	85	62
3-Bromo-2-nitrobenzoic acid	59	62
Malachite Green "G"	85	58
Sodium 2,6-dichloroindophenol	85	58
2-Hydroxy-1,3,2-benzodioxastibiole	65	53

(a) This is Eastman's product T-2246 which consists of 65 per cent of the 1,5-isomer and 35 per cent of the 1,8-isomer.

(b) Courtesy of Dr. Samuel Clark, University of Mississippi.

(c) Courtesy of Dr. David Shirley, Tulane University.

ard test chambers using more critical exposures of 30 minutes and residual effectiveness was determined over a period of several weeks.

RESULTS

Some 2,400 compounds have been screened to determine their potential usefulness as synergists for DDT in residual deposits. Of these compounds, 17 chemicals (table 1) have been approximately equal to or better than DMC. The resistance of the field strain of *M. domestica* varied considerably, to the standard DDT-DMC, and the comparative mortalities from this combination have been listed for each compound.

Limited laboratory tests have been made in standard test chambers with residual deposits of eight of the more promising synergistic combinations. The synergistic combinations were applied as xylene emulsions on plywood panels to give residual deposits of 200 mg. of DDT combined with 200 mg., 40 mg., and 20 mg. of the test chemical per sq. ft., respectively. The deposits were tested four weeks after spray application using a more critical exposure period of 30 minutes. Under these test conditions only three of the synergistic compounds have continued to show promise (table 2). Dihydroxyanthraquinone, hydroxy-pentamethyl flavan, *p*-chlorobenzaldehyde semicarbazone, phenylmercuric salt of 2,4-pentanedione, and 3-hydroxy-2-naphthoic acid combined with DDT gave less than 10 per cent mortality in all ratios under the test conditions.

TABLE 2

Twenty-four-hour mortalities of adult M. domestica *after 30-minute exposures to various 4-week-old combined deposits of DDT and test synergists*

TEST COMPOUND	DDT: TEST COMPOUND (MG./SQ. FT.)	24-HR. MORTALITY (PER CENT)	
		M	F
p-Chlorophenyl 1,2-dichloro-2-(*p*-chlorophenyl)-ethyl ketone	200:200	100	100
	200:40	97	86
	200:20	100	95
1,1-bis(*p*-Chlorophenyl)-ethanol (DMC)	200:200	89	71
	200:40	88	68
	200:20	5	8
1,1-bis(*p*-Chlorophenyl)-ethane	200:200	99	81
	200:40	29	2
	200:20	1	2

DISCUSSION

Compounds from several chemical series showed activity as DDT synergists against DDT-resistant house flies and this may indicate that more than one blocking mechanism is possible. One of the best supported theories for the mechanism of resistance to the action of DDT and its analogs is based on a reaction involving the loss of hydrogen halide from the molecule and the formation of 1,1-bis(*p*-chlorophenyl)-2,2-dichloroethylene (DDE). If the dehydration of 1,1-bis(*p*-chlorophenyl)-ethanol (DMC) occurred it would give 1,1-bis(*p*-chlorophenyl)-ethylene, a compound closely analogous to DDE. If the dehydrogenation of 1,1-bis(*p*-chlorophenyl)-ethane occurred it would likewise give 1,1-bis(*p*-chlorophenyl)-ethylene. If these reactions were competing with the detoxification mechanism at the foci of their activity in the insect, the DMC or the 1,1-bis(*p*-chlorophenyl)-ethane might thus afford protection for the DDT. The chlorinated chalcone, *p*-chlorophenyl 1,2-dichloro-2-(*p*-chlorophenyl)-ethyl ketone may undergo dehydrohalogenation and offer protection to the DDT in this manner.

Since the arrangement and proportions of the DDT and the potential synergist chemical on the surface of the residual deposits may play an important part in the synergistic action of the combination, further investigations of those chemicals showing promise in rapid screening will be made. Other types of formulations such as suspensions, solutions in solvents other than xylene, and others will be evaluated using the more critical evaluation factors of 30-minute exposures to deposits 4 weeks after spray application.

SUMMARY

Rapid screening tests have been made with combined residual deposits of 200 mg. of DDT and 20 mg. of test compound per sq. ft. to determine any synergistic action against DDT-resistant house flies. Of 2,400 compounds tested, 17 appeared about equal to or better than 1,1-bis(*p*-chlorophenyl)-ethanol (DMC).

More critical laboratory tests on 8 of the 17 compounds with 4-week-old residual deposits and 30-minute exposure periods revealed three promising compounds: (a) *p*-chlorophenyl 1,2-dichloro-2-(*p*-chlorophenyl)-ethyl ketone; (b) 1,1-bis(*p*-chlorophenyl)-ethanol, and (c) 1,1-bis(*p*-chlorophenyl)-ethane.

Further evaluations with different formulations will be made with the 17 compounds showing promise in the rapid screening tests.

REFERENCES

Bruce, W. N. 1949. Latest report on fly control. Pest Control, **17:** 7, 28.

Deonier, C. C.; Cain, T. L., Jr.; and McDuffie, W. C. 1950. Aerial spray tests on adult salt-marsh mosquitoes resistant to DDT. J. Econ. Ent., **43:** 506–510.

King, W. V.; and Gahan, J. B. 1949. Failure of DDT to control house flies. J. Econ. Ent., **42:** 405–409.

Knipling, E. F. 1950. Insecticidal resistant flies and mosquitoes. Proc. Chem. Specialties Mfrs. Assoc. June: 87–88.

March, Ralph B.; and Metcalf, R. L. 1949. Laboratory and field studies of DDT resistant houseflies in southern California. Calif. State Dept. Agr. Bul., **38:** 93–102.

March, Ralph B.; and Metcalf, R. L. 1949a. Development of resistance to organic insecticides other than DDT by houseflies. J. Econ. Ent., **42:** 990.

Missiroli, A. 1947. Riduzione o eradicagione degli anofeli. Riv. di Parassitol., **8:** 141–169.

Mosna, E. 1951. Sulla resistenza delle mosche domestiche al chlordane. Riv. Parassitol., **12:** 27–36.

Perry, A. S.; and Hoskins, W. M. 1950. The detoxification of DDT by resistant houseflies and inhibition of this process by piperonyl cyclonene. Science **111:** 600–601.

Pratt, John J., Jr.; and Babers, Frank H. 1950. Cross tolerances in resistant house flies. Science **112:** 141–142.

Sternberg, James; Kearns, C. W.; and Bruce, W. N. 1950. Absorption and metabolism of DDT by resistant and susceptible houseflies. J. Econ. Ent., **43:** 214–219.

Sumerford, Wooten T.; Goette, Mary B.; Quarterman, Kenneth D.; and Schenck, Sheely L. 1951. The potentiation of DDT against resistant house flies by several structurally related compounds Science **114:** 6–7.

Wiesmann, R. von. 1947. Untersuchungen uber das physiologische Verholten von *Musca domestica* L. verschiedener Provenienzen. Schweiz. Ent. Gesell. Mitt., **20:** 484–504.

RESUMEN

Lleváronse a cabo pruebas rápidas de selección usando depósitos residuales compuestos de 200 miligramos de DDT y 20 miligramos por pie cuadrado del compuesto

en prueba para determinar cualquier acción sinergística contra moscas de casa resistentes a DDT. Diecisiete de los, 2,400 compuestos probados resultaron igual o mejor que "1, 1-bis(*p*-chlorophenyl)-ethanol."

Pruebas de laboratorio más críticas llevadas a cabo en 8 de los 17 compuestos usando depósitos residuales de cuatro semanas de edad y exposiciones de 30 minutos revelaron tres compuestos prometientes: "(a) *p*-chlorophenyl 1,2-dichloro-2-(*p*-chlorophenyl)-ethyl ketone; (b) 1,1-bis(*p*-chlorophenyl)-ethanol," y "(c) 1,1-bis(*p*-chlorophenyl)-ethane."

Futuras valuaciones con formulaciones diferentes harán uso de los 17 compuestos prometientes en las pruebas rápidas de selección.

ANOPHELES AZTECUS, MALARIA, AND MALARIA CONTROL IN THE VALLEY OF MEXICO

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Since most of the information concerning the characteristics of endemic malaria in the Valley of Mexico, including the role of *Anopheles aztecus* Hoffmann, 1935 as vector, epidemiological studies and report of the recent control campaign, have appeared in the Mexican malaria literature only, a review of the subject is presented herewith for readers of English.

The Xochimilco-Mixquic region of the Valley of Mexico is well-known to tourists, lying from 10 to 30 miles to the south of the city of Mexico. At the time of the conquest of Mexico, the region was covered by a lake, with the present towns on the margins, but over the centuries, particularly with changes introduced by the drainage canal which drains the old lakes of the Valley of Mexico into the Rio Moctezuma, a tributary of the Rio Panuco which empties into the Gulf of Mexico, the region has been drying up. At the present time, there is a maze of hundreds of kilometers of canals in the Xochimilco region, some only two to three feet wide, dug to bring water near the fields, and others several yards, up to 30 yards wide, and open for the traffic of flat-bottomed scows and dugout canoes. On Sundays and holidays, the canals are crowded with hundreds of flower-festooned boats, full of tourists, orchestras, food, flower and curio vendors, and photographers. The region is now a productive truck gardening and flower growing section. The fields, called chinampas, are fertilized by scooping up bottom mud from the canals and spreading it over the land. The narrower canals become clogged with vegetation, mostly *Eichornia*, and this is often cut and spread over the fields as fertilizer and binder for the mucky soil. Water vegetation is also harvested and used to feed livestock. Willow trees (*Salix humboldtiana*) line the banks of the canals, and serve to hold the soil. These narrow, straight willows, much resembling Lombardy poplars, give a characteristic appearance to the entire region.

The population of the malarious towns of the region is approximately 40,000, although the population living in the parts of the towns near the water, and most likely to be affected by malaria, probably does not exceed 20,000. The inhabitants are of mixed ancestry, with a marked preponderance of Indian blood, of the old Xochimilca and Aztec stocks. Until a few years ago, the Indian language, Nahuatl, was spoken in many of the communities, but of recent years, Spanish is almost universally spoken, and only a few of the oldsters still converse in the Indian tongue. Although the region is not a poor one, with many earning a living from the good soils, the standards of living are still very low. In each community, there are a few well-constructed houses of brick or adobe with cement and plaster walls and tile roofs, but also a large number

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of very humble dwellings, of adobe or of rock, with straw roofs, and even poorer houses of cornstalks. The poorer houses are found particularly in the peripheral zones of the towns. Sanitary facilities are almost universally lacking, although all of the towns have piped potable water supply, with public faucets. Bustamante (1939) presents data about the general disease conditions of the region in the period 1936 to 1938. General mortality rate was 33.91 per thousand. Infant mortality rates were high. Pneumonia and bronchopneumonia were leading causes of death, outbreaks of epidemic typhus fever were not uncommon in the past, and outbreaks of measles and whooping cough exacted a heavy toll on the young. Malaria was distinctly a minor cause of mortality in the region. Although there has been some improvement in conditions in the intervening decade, the general picture has not changed appreciably. The modern new health center established in Xochimilco to serve the region will probably help greatly to better conditions in the coming years.

The Xochimilco region of the Valley of Mexico is at an altitude of 7,426 feet above sea level. The mean summer temperature is 61° F. and the mean winter temperature, 48° F. Frosts are not infrequent in the fall and winter months, and at times, the smaller, quiet canals will freeze over at night with a thin layer of ice which melts during the day. There are two well-defined seasons: the wet, extending from late May or June into September and at times early October, and the dry, from October to May or June. Total rainfall figures (from a station at Xochimilco, maintained from 1933 to 1940) vary from 1,360 mm. to 2,364 mm., with maximum monthly rainfalls in the rainy season of 400 mm. and minimum monthly rainfalls during the dry season of 15 mm. Relative humidity varies from a maximum in August, September, and October of 75 per cent to a minimum of about 45 per cent in February and March. (These values are computed from monthly means of 24 readings daily, data furnished by the Servicio Meteorológico de la Secretaría de Agricultura.)

For many years, malaria has been recognized as being endemic in the Valley of Mexico. Bustamante (1939) presents an extensive epidemiological and entomological study, and while later investigations have filled in lacunae in his data, they have not changed his concepts. This reference carries a bibliography of earlier studies, which will not be duplicated here. Using data collected from the health unit in Xochimilco, all malaria cases being confirmed by laboratory examination, Bustamante shows that *Plasmodium vivax* is the only endemic species of parasite present, and that there is a peak of cases in the fall months. Table 1, reproduced from his paper, presents clinic data:

TABLE 1

Confirmed cases of malaria (P. vivax) *seen in the clinic in Xochimilco, D.F.*

YEAR	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
1935	2	1	3	2	3	7	7	25	39	79	141	55	364
1936	20	11	15	17	27	32	44	50	47	94	41	33	431
1937	21	19	17	31	58	82	120	129	129	210	129	100	1,045

It can be seen that a considerable malaria problem existed in the region. More recent reliable data do not exist, although data from the health clinic maintained by

the Cruz Blanca, in San Gregorio Atlapulco, covering the period 1942 to 1946, indicate that they treated between 200 and 750 cases of clinically diagnosed malaria annually in this one town alone. There can be no doubt that the figures cited represent only a small fraction of the malaria cases occurring annually in the region, since several of the most malarious towns are 20 to 30 miles from the health center, and it is certain that very few people traveled this far for treatment.

Malariological investigations were undertaken by us in the towns of the region, in 1947 and 1948, splenometric and parasitologic examinations being carried out on school children between the ages of 5 and 15, in the spring and fall of each year. Even in this brief period, it is evident that seasonal and annual fluctuations in rates may be considerable, as can be seen in Tables 2 and 3. With malaria of low endemic-

TABLE 2

Spleen and parasite rates—Xochimilco-Mixquic region, 1947–1950

TOWN	BEFORE SPRAYING WITH INSECTICIDE												15 MONTHS AFTER SPRAYING WITH INSECTICIDE		
	Spring 1947, 515 children examined			Autumn 1947, 986 children examined			Spring 1948, 937 children examined			Autumn 1948, 790 children examined			Autumn 1950, 838 children examined		
	Spleen rate	Parasite rate	AES	Spleen rate	Parasite rate	AES	Spleen rate	Parasite rate	AES	Spleen rate	Parasite rate	AES	Spleen rate	Parasite rate	AES
		per cent		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*				
Mixquic*	?	6.0	?	18.5	5.7	1.3	21.6	2.4	1.55	11.4*	4.5*	1.3*	7.0	0.0	1.07
Tlaxialtemalco†	16.0	4.0	1.5	18.5	7.1	1.3	24.0	4.2	1.36	23.8†	2.5†	1.2†	3.3	0.0	1.0
Tetelco	?	19.0	?	7.5	0.0	1.0	12.2	0.0	1.2	29.5	4.5	1.1	3.3	0.0	1.0
Atlapulco	?	0.0	?	20.0	7.3	1.2	17.5	0.5	1.34	17.5	4.5	1.1	8.6	0.0	1.14
Acalpixca	?	7.2	?	19.0	1.0	1.3	14.5	0.0	1.3	30.9	0.8	1.2	5.2	0.0	1.0
Nativitas	?	7.2	?	18.0	0.7	1.2	15.5	0.5	1.6	14.2	0.8	1.2	6.3	0.0	1.0
Totals	?	8.6	?	18.2	4.1	1.22	17.4	1.1	1.4	18.7	3.2	1.2	6.7	0.0	1.07

* Sprayed in the summer of 1948 and 1949 with DDT.

† Sprayed in the summer of 1948 and 1949 with gammexane.

The remaining towns were sprayed with DDT in the summer of 1949.

ity, it was considered necessary to collect all the information possible before initiating control work, in order to be able to evaluate more precisely the effects of control work. Consequently, in most instances, four examinations were carried out before beginning control operations. In all of these examinations, only *P. vivax* was encountered. Overall spleen rates varied from 17 to 18 per cent and overall parasite rates from 1.1 per cent in the spring of 1948 to 8.6 per cent in the spring of 1947, the last figure before control work began being 3.2 per cent in the fall of 1948.

Bustamante mentions encountering four infections of *P. falciparum*, and in 1949 there was a small *P. falciparum* outbreak of three cases in Santa Cruz Acalpixca. Such small outbreaks probably occur sporadically when a gametocyte carrier enters the region during one of the warmer months of the year, but it is evident that *P. falciparum* has not been able to establish itself successfully.

Beltran *et al.* (1948), in a study of the clinical characteristics of a *P. vivax* strain

isolated from a patient in Xochimilco, and passaged to neurosyphilitics, concluded that it did not behave differently clinically from other *vivax* strains previously studied by them. The relapse pattern has never been determined. However, there is no epidemiological evidence to indicate a protracted incubation period such as has been noted in temperate-region malaria in Holland.

The entomological aspects of malaria transmission are relatively simple in that only two possible malaria vectors are present, namely, *A. aztecus* Hoffmann, 1935 and *A. pseudopunctipennis* Theobald, 1901. Here it may be mentioned that Dampf, until his death, maintained that *A. aztecus* was a synonym of *A. occidentalis* Dyar and Knab 1906. Recent taxonomic and systematic studies, including those of Vargas (1941, 1943, and 1944) accord full specific status to *aztecus*. Pelaez (1945) and Aitken (1945) continue to refer to the species as *A. maculipennis aztecus*. It is interesting to note that during the course of laboratory studies in which the growth of the egg of *A. aztecus* after hatching is described (Downs, 1951), an unusual egg variant was

TABLE 3

Comparison between spleen sizes and parasite rates for the Xochimilco-Mixquic region, 1947 to 1950 (spleen size—Hackett)

SPLEEN SIZE	BEFORE SPRAYING WITH INSECTICIDE								AFTER SPRAYING WITH INSECTICIDE	
	Spring 1947		Autumn 1947		Spring 1948		Autumn 1948		Autumn 1950	
	No. slides taken	Per cent positive	No. slides taken	Per cent positive	No. slides taken	Per cent positive	No. slides taken	Per cent positive	No. slides taken	Per cent positive
0	240	4.2	190	3.2	188	1.05	174	1.7	194	0.0
1	61	8.2	160	3.8	132	0.75	147	2.7	52	0.0
2	20	15.0	44	13.6	64	0.0	31	19.0	4	0.0
3	2	50.0	3	33.3	9	11.1	0	—	0	—
4	0	—	0	—	0	—	0	—	0	—
5	0	—	0	—	0	—	0	—	0	—

Note: All examinations were thick-drop. All positives were *P. vivax*.

encountered in two only of thousands of egg batches examined. All of the eggs of each of these batches had a patterned exochorion, resembling the patterns seen in some of the members of the European *maculipennis* complex. These eggs were fertile and hatched (Downs, 1950).

Hoffmann in 1929 considered the possibility of *A. pseudopunctipennis* and *A. aztecus* (called by him at this time *A. quadrimaculatus*) as vectors in the Valley of Mexico, and attached most importance to *A. pseudopunctipennis*, a well-recognized vector in other parts of Mexico. However, the same author in 1936 considered *A. aztecus* to be the undoubted vector of malaria in the region of Lake Patzcuaro, Michoacan. Bustamante in 1939 had already declared, after giving details of extensive mosquito collecting and field observations, that *A. aztecus* was undoubtedly the vector of malaria in the Xochimilco region.

House captures of anophelines were made by Bustamante in 1935–36 and by us in 1947–48. These data are summarized in Table 4.

Since *A. pseudopunctipennis*, wherever encountered in Mexico, rests commonly in houses throughout the day, the observations force the conclusion that the species is so rarely encountered in the region that it cannot function adequately as a vector to maintain the degree of endemic malaria observed. Furthermore, it is only occasionally encountered in the larval stage, usually in conjunction with algal mats, and this habitat is so characteristic and so easily seen and checked, that it is highly improbable that any considerable larval densities have passed unobserved. Therefore, *A. aztecus* is, by exclusion, the principal vector of malaria in the region.

TABLE 4

House captures of A. aztecus *and* A. pseudopunctipennis *in the Xochimilco region*

OBSERVER	YEAR	*A. aztecus*	*A. pseudopunctipennis*
Bustamante (1939)	1935–36	1231	2
Bordas and Downs (1950)	1947	2593	16
Bordas and Downs (1950)	1948	1438	0

In dissections of *A. aztecus* made in 1947 to 1948 (Downs and Bordas, 1949), one specimen was encountered with an oocyst infection of the gut, and one specimen with an oocyst infection of the gut and sporozoites in the salivary glands. A total of 613 mosquitoes, all captured in houses, were dissected. Salivary glands were examined in 611 of these, and stomachs in 411.

Sandoval *et al.* (1950) report the successful infection of *A. aztecus* with both *P. vivax* and *P. falciparum*, and succeeded twice in transmitting *P. vivax*. *A. aztecus* was infected readily with several strains of *P. vivax* and one strain of *P. falciparum*, but apparently is less susceptible than the National Institutes of Health strain of *A. quadrimaculatus* in parallel feedings. Table 5, modified from the table in Sandoval's paper, presents these data:

TABLE 5

Comparative susceptibility of A. aztecus *and* A. quadrimaculatus *to strains of* P. vivax *and* P. falciparum

MALARIA STRAIN	NUMBER OF PARALLEL INFECTIVE FEEDINGS	*A. aztecus*			*A. quadrimaculatus*		
		Number dissected	Number infected	Per cent infected	Number dissected	Number infected	Per cent infected
P. vivax #11 Xochimilco	11	105	32	30	166	69	42
P. vivax #16 Tacambaro, Mich.	2	28	4	14	64	13	20
P. vivax #17 Huetamo, Mich.	6	86	25	29	201	87	43
Totals P. vivax	19	219	61	28	431	169	39
P. falciparum #15 Veracruz	6	82	12	15	147	53	36

A more detailed breakdown of these figures, in the original paper, illustrates clearly that this difference in susceptibility follows through all of the parallel feedings, and shows that the differences observed are not caused by the vagaries of chance, in which a single highly infective feeding, where many individuals of one species and few of the other were fed, could produce a considerable imbalance in final rates.

Bustamante describes the breeding places of *A. aztecus*, mentioning particularly quiet waters of the canals not subject to boat traffic, and remarks that the larvae can be found throughout the winter, even under the thin ice which occasionally forms. A later detailed study of larval ecology by Bordas and Downs (1950) points out that the larvae are found most commonly in clear water with abundant emergent or submerged vegetation. Surface mats of *Potamageton* and of *Ceratophyllum* and associations of *Nitella-Ceratophyllum* are favored breeding sites. Floating vegetation (*Lemna* and *Azolla*) does not constitute a favorable habitat. Wherever even a small fraction of the water surface is covered by either of these two plants, it is rare that larvae are found, even in small numbers. Dense mats of *Eichornia* and of *Pistia* are also

TABLE 6

House captures of A. aztecus, *region of Xochimilco, D.F.*

YEAR	NUMBER OF HOUSE CAPTURES	ANOPHELINES ON CEILINGS		ANOPHELINES ON WALLS		ANOPHELINES UNDER BEDS		TOTAL ANOPHELINES CAPTURED	AVERAGE NUMBER PER HOUSE
		number	per cent	number	per cent	number	per cent		
1947	275	252	9.7	1741	67.1	600	23.1	2593	9.4
1948	148	89	6.2	998	69.4	351	24.4	1438	9.7
Totals.....	423	341	8.5	2739	67.9	951	23.6	4031	9.5

poor breeding areas. Larval densities are almost always low, even in favorable habitats, and in many cases, what appear to be favorable habitats will have no larvae. Occasionally, high larval densities are encountered, even as high as 1000 larvae per square meter, particularly on *Potamageton* mats. Although extensive breeding areas, to all outward appearances favorable, exist in the region, breeding is on the whole very spotty, leading to the impression that there exist potent limiting factors to the multiplication of *A. aztecus* in the region.

Adult mosquitoes are found in houses throughout the year, but are few (average two per house) in the dry spring months, rising to an average of 21 per house in the wetter late summer and fall months. Table 6 presents results of house captures.

A. aztecus was commonly found on walls and underneath the crude low-board beds in common use. Several houses were encountered which were consistently good "mosquito houses." In one house in Tetelco, approximately 100 mosquitoes could be found on each visit during the fall months of the year. These houses were no different apparently from other houses in the same locality, which consistently harbored fewer mosquitoes. It would be important to know if these "mosquito houses" were also "malaria houses," but no data could be obtained on this point.

Numerous searches were made for mosquitoes resting out of doors. Small numbers

were found under the few bridges in the region. Night captures in semi-open cattle stables yielded only small numbers of anophelines, and daytime captures in such localities yielded no mosquitoes. Captures in an Egyptian-type stable trap with a goat as bait, and on several occasions with human bait, yielded an average of less than one *A. aztecus* per night. Precipitin testing for source of blood meals has not been carried out with this species. However, it has repeatedly been observed feeding on man, even in daytime, and less frequently observed feeding on animals. It is probable that it feeds by preference on man.

There is a rather close correspondence between the curve of adult anopheline density and the rainfall curve, as pointed out by Bustamante. The increase in surface water collections at the beginning of the rainy season in June, however, does not directly affect *A. aztecus*, which does not usually breed in small temporary water collections. *Aedes*, *Culex*, and *Culiseta* densities rise markedly at this time. However, the rainy season also brings a rise in relative humidity, which reaches its maximum in August, September, and October, with values of 75 per cent (contrasted with a minimum of about 45 per cent in February and March). Therefore, rather than the rains directly, the months of highest relative humidity coincide with those of maximum anopheline density.

A. aztecus was colonized by Downs *et al.* (1948) and the colony has been maintained to date. The species develops slowly at temperatures between 72 and 79° F., requiring 29 to 30 days for a complete cycle, whereas *A. quadrimaculatus* and *A. albimanus*, under the same conditions, complete their development in about half the time. Probably this slow development, coupled with unfavorable temperature factors in the winter, acting on the larvae, and unfavorable humidity factors in the spring and early summer, acting on the adults, helps to explain why *A. aztecus* is not more abundant in the region.

The house-frequenting habits of the adult female mosquitoes suggested that a program of DDT residual house spraying should be effective in controlling malaria in the region. Accordingly, in 1948, the houses and outbuildings, stables, and sheds of the town of Mixquic were sprayed with a suspension of DDT water-wettable powder at a rate of 200 mgm. of DDT per square foot, and in San Luis Tlaxialtemalco, with gammexane P 520 (furnished by Imperial Chemicals Company, Ltd.) at a rate of 20 mgm. of the gamma isomer of benzene hexachloride per square foot. Entomological checks made after this trial spraying indicated that good control, as measured by counts of house-resting anophelines, resulted. No mosquitoes were seen for a full year after treatment in the DDT-treated village, and very few until after eight months in the gammexane-treated village.

In 1949, a program was carried out by the health authorities of the Federal District, under the direct supervision of Dr. Enriquez Chavez. Only those houses in the two to three blocks nearest the water were sprayed in the towns of Xochimilco, Nativitas, Santa Cruz Acalpixca, San Gregorio Atlapulco, Tlahuac, San Luis Tlaxialtemalco, Tetelco, and San Andres Mixquic. A total of 5,421 houses were sprayed and 2,900 kilograms of DDT 50 per cent wettable powder were used. The total cost of the project, including DDT, salaries and wages, transportation, and repair of equipment, was 26,600 pesos ($3,075.00 U. S.).

A malaria resurvey, carried out 15 months after the original spraying, revealed (see Tables 2 and 3) that excellent control had been achieved (Downs *et al.*, 1950). Indices of splenic enlargement were much lower and the average enlarged spleen was smaller. No parasite-positive children were encountered. (Of 838 children examined, thick drops were taken on all of the 56 spleen-positive children, and on 194 of the 782 spleen-negative children). Dr. Guevara Rojas, the head of the health unit in Xochimilco, further strengthened these findings, stating that in 1950, they had had only two confirmed cases of malaria in the health unit, both of these cases in migrant laborers who had entered the region recently from a malarious neighbor state. In the Cruz Blanca clinic in San Gregorio it was stated also that very few clinical cases of malaria had been seen during 1950.

Recommendations for future plans for malaria control work have been submitted to the health authorities of the Federal District. Studies on the adobe of the Xochimilco-Mixquic region (Downs *et al.*, 1951) have shown that this adobe contains very little iron and decomposes DDT very slowly in the test for catalytic decomposition. Biological tests with mosquitoes indicate that a DDT residual deposit will retain a high degree of effectiveness for three years. Consequently, it has been recommended that a repeat spraying program be carried out two years after the 1949 spraying. Following this, it is suggested that the health unit in Xochimilco maintain vigilance over the region, and that future sprayings be carried out only when cases of autochthonous malaria begin to appear in one or another town of the region. At such time, it is considered advisable that all areas previously sprayed be re-sprayed.

The above program, if properly supervised, should give economical and effective control of malaria to this region.

SUMMARY

1. Malaria is endemic in the Xochimilco-Mixquic region of the Valley of Mexico at an altitude of 7,426 feet above sea level. *P. vivax* is the only species of endemic malaria. *A. aztecus* Hoffmann, 1935 has been found naturally infected in the region, can be readily infected in the laboratory, and has been shown to be able to transmit *P. vivax*. The species is commonly found in houses in the region and bites man freely. Basing judgment upon accumulated field and laboratory experience, it is considered as the sole malaria vector of importance in the region.

2. A malaria control program consisting of spraying of all houses and other buildings in the zones of the towns near the breeding areas, with DDT suspension made up from 50 per cent water-wettable powder, applied at the rate of 200 mgm. per square foot, has given, up to the present, excellent control of malaria. Parasite rates have dropped to zero and spleen rates have fallen markedly.

3. Control recommendations for the future are to re-spray after a two-year interval, and after that, to re-spray only when indicated by appearance of autochthonous cases of malaria.

REFERENCES

Aitken, T. H. C. 1945. Studies on the anopheline complex of western America. Univ. of Calif. Publ. Entomology, **7**: 273–364.

Beltrán, E. and Villasana, L. 1948. El paludismo en el Distrito Federal. Características de una cepa de *Plasmodium vivax* aislada en Xochimilco. Rev. Inst. Sal. Enf. Trop., **9**: 21–25.

Bordas, E. and Downs, W. G. 1950. Estudio ecológico de *Anopheles aztecus* Hoffmann 1935. Rev. Inst. Sal. Enf. Trop., **11:** 125–132.

Bustamante, M. E. 1939. Epidemiología del paludismo en el sur del Valle de México, zona de Xochimilco. Gac. Med. Mex., **69:** 1–31.

Downs, W. G. 1950. Una variante rara de huevecillo de *Anopheles aztecus* Hoffmann 1935. Rev. Inst. Sal. Enf. Trop., **11:** 175–177.

Downs, W. G. 1951. Growth changes of anopheline eggs in water and in saline solutions. J. Nat. Malaria Soc., **10:** 17–22.

Downs, W. G. and Bordas, E. 1949 Hallazgo de *Anopheles aztecus* naturalmente infectados con *Plasmodium* en la región de Xochimilco. Rev. Inst. Sal. Enf. Trop., **10:** 321–325.

Downs, W. G., Bordas, E., and Arizmendi, A. 1948. Colonización en el laboratorio de *Anopheles aztecus* Hoffmann. Rev. Inst. Sal. Enf. Trop., **9:** 175–176.

Downs, W. G., Bordas, E., and Enriquez Cháves, A. 1950. El control del paludismo en la Región de Xochimilco, D. F. Rev. Inst. Sal. Enf. Trop., **11:** 99–105.

Downs, W. G., Bordas, E., and Navarro, L. 1951. Duración de la efectividad de depósitos residuales de DDT en superficies de adobe. Medicina (Rev. Mex.), **31:** 215–220.

Hoffmann, C. C. 1929. Los mosquitos *Anopheles* transmisores de paludismo en el Valle de México. Bol. Depto. Salub. (Mexico), **2:** 11–23.

Hoffmann, C. C. 1936. Nota sobre el anofelismo en el centro de Michoacan. Bol. Inst. Hig. Med. (Mexico), **2:** 6, 330–376.

Pelaez, D. 1945. Anofelinos de México. I. Clave para la determinación de especies y subespecies basada en los caracteres de las hembras adultas. Ciencia, **6:** 69–77.

Sandoval, A. M., Beltrán, E., and Downs, W. G. 1950. Nota sobre la infección experimental del *Anopheles aztecus* Hoffmann. Rev. Inst. Sal. Enf. Trop., **11:** 149–152.

Vargas, L. 1941. Detalles morfológicos de los *Anopheles* americanos del grupo *maculipennis* y especies próximas. Ciencia, **2:** 23–25.

Vargas, L. 1943. El grupo *maculipennis* en el nuevo mundo y el *Anopheles earlei*. Rev. Inst. Sal. Enf. Trop., **4:** 279–286.

Vargas, L. 1944. Algunas consideraciones sobre *Anopheles occidentalis* Dyar and Knab 1906. Rev. Inst. Sal. Enf. Trop., **5:** 215–219.

RESUMEN

1. La malaria se muestra endémica en la región Xochimilco-Mixquic del Valle de Méjico a una altura de 7,426 pies sobre el nivel del mar. *Plasmodium vivax* es la única especie de la malaria endémica. *Anopheles aztecus* Hoffmann, 1935 se ha encontrado infectado naturalmente en esta región, puede infectarse fácilmente en el laboratorio y puede transmitir *Plasmodium vivax*. Esta especie comúnmente se encuentra en las casas de esta región y pica al hombre espontáneamente. Juzgando por la experiencia adquirida en el campo y en el laboratorio está considerado como el único vector de malaria de importancia en esta región.

2. Un programa de control de malaria consistente en rociar las casas y otros edificios en las zonas de los pueblos cerca de las áreas de crianza, con una suspensión de DDT compuesta de 50 por ciento de polvo humediceble con agua aplicada en una proporción de 200 miligramos por pie cuadrado ha demostrado hasta ahora control excelente contra malaria. La proporción de parásitos descendió a zero y las proporciones esplénicas han disminuído grandemente.

3. Las recomendaciones para el control futuro hacen uso de nuevas rociadas después de un intervalo de dos años, y después, solamente cuando la presencia de casos indígenos de malaria así lo indiquen.

THE COURSE OF THE BLOOD-INDUCED *PLASMODIUM BERGHEI* INFECTION IN WHITE RATS

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Vincke and Van den Bulcke (1949) showed the white rat, *Rattus rattus*, to be highly susceptible to infection with *Plasmodium berghei*. These authors induced infections by the intraperitoneal inoculation of parasitized blood; the parasitemia, which became evident after two or three days, increased progressively to a peak resulting in death to approximately 50 per cent of the animals. Corradetti (1950) described a period of immunity following the acute attack in recovered animals which protected against reinfection with a homologous strain for as long as 205 days. Raffaele and Baldi (1950), Baldi (1950), and Galliard and Lapierre (1951) have described the infection in the white rat and have associated the severity of the infection with the age of the animals. Baldi sought to explain this phenomenon on the basis that the young rats had a high proportion of reticulocytes for which the parasites show a decided affinity. In their studies on the relationship between the parasite and the cells of the erythrocyte series, during the course of a primary attack in the white rat, Corradetti and Verolini (1951) have also described a marked tendency of the parasite to invade the immature erythrocytes. In the tree rat, *Thamnomys surdaster*, Vincke and Van den Bulcke (1949a) have described an infection similar to that of the white rat but with lower infection and mortality rates; recovered animals generally exhibited chronic infections. Rodhain (1949) observed a low parasitemia of short duration in the cotton rat, followed by recovery.

In this laboratory the course of the blood-induced *Plasmodium berghei* infection has been previously reported only in the white mouse, *Mus musculus* (Mercado and Coatney, 1951). The work reported in this paper deals with a similar study in the white rat, *Rattus rattus*, in which the similarities and differences between the two types of infection and the possible relationships to the findings reported by earlier investigators are discussed.

MATERIALS AND METHODS

In these investigations the KBG 173 strain of *P. berghei* was used. This strain was kindly furnished to us by Dr. A. Dubois, Director of the Prince Leopold Institute of Tropical Medicine, Antwerp. It has been maintained in this laboratory by blood passage.

Three sets of 16 rats each were studied; each animal was approximately six weeks old. Each rat received intravenously approximately 5,000 parasitized red blood cells. After inoculation the animals were examined daily for physical signs indicative of malarial infection. Beginning on the second day after inoculation, blood smears

* Grateful acknowledgment is extended to Miss Nancy Allen for technical assistance in this work and in the work covered by our previous paper on *P. berghei*.

were taken daily during the entire course of the infection. The blood smears were stained with Giemsa. Because of the common occurrence of multiple infections, the parasitemia was expressed as the percentage of parasitized red blood cells rather than according to the actual number of parasites present. (The parasitemia was also recorded as the true number of infected red blood cells, based on the percentage of parasitized red cells and on the erythrocyte count in millions per cu. mm. Thus, an average per cent parasitemia of 7.00 ± 0.61 on the second day of patency and an average erythrocyte count of 4.04 ± 0.57 accounted for 282,800 infected red cells.) A record also was kept of the occurrence of segmenters and, whenever counting was facilitated by a distinct organization of merozoites, the number of merozoites produced per segmenter.

In order to ascertain the degree of anemia produced during the infection, red blood cell counts were made just before inoculation and generally, daily during the infection.

The length of an observation period expressed in days is relative to the number of complete days following the day in which the event took place. Thus, a patent period of ten days refers to ten complete days following the day on which the animals first became positive.

EXPERIMENTAL RESULTS

Of the 48 rats inoculated 47 (98 per cent) became infected. Of the infected animals, two were injured accidentally and died early in the infection. Of the 45 animals remaining, 40 died as a result of the infection and 5 recovered.

Among those animals which died, the first parasites appeared in the blood 2 to 5 days after inoculation; 7 (17.5 per cent) animals first exhibited parasites on the second post-inoculation day, 17 (42.5 per cent) on the third, 12 (30 per cent) on the fourth, and 5 (10 per cent) on the fifth. The mean prepatent period was 3.33 ± 0.15 (S.E. of mean) days. The parasitemia increased progressively during the first 15 days of patency; the greatest percentage of animals reached the peak in 10 to 15 days (mean, 11.2 ± 0.5). The average daily parasite counts showed a steady increase from the onset of parasitemia until the fifteenth day when 62.7 ± 3.9 per cent of the red blood cells were infected. However, when the parasitemia was expressed as the true number of red blood cells parasitized rather than as the per cent parasitemia, this increase appeared more gradual. In the former, a three-fold increase from 282,800 parasitized red cells on the second patent day to 834,520 on the eighteenth day was observed; in the latter, a ten-fold increase occurred from 7.00 ± 0.61 to 67.3 ± 7.84 for the corresponding intervals (figure 1). After the peak the parasitemia showed little change until death, except for a slight decrease and increase on the sixteenth and eighteenth days, respectively (figure 1). The patent period varied from 8 to 21 days (mean, 12.23 ± 0.53). Death of 25 animals (62.5 per cent) occurred within 24 hours after their peak parasitemia, 9 (22.5 per cent) died in two days, 3 (7.5 per cent) in three, 2 (5 per cent) in four, and one (2.5 per cent) in five days.

In those animals which survived the acute infection the first parasites appeared in the blood 2 to 5 days after inoculation; the mean prepatent period was 3.2 ± 0.5

days. The parasitemia increased progressively during the first eight days of patency, the peak was reached in 7 to 16 days (mean, 11.0 ± 1.64). At the peak 22.0 ± 3.58 per cent of the red blood cells were parasitized, thereafter a gradual decrease occurred which was interrupted only by small increases on the sixteenth and eighteenth days. The patent period, i.e. the number of days from the onset of parasitemia until the animals first became smear negative, varied from 16 to 22 days (mean, 18.6 ± 1.18) (figure 2).

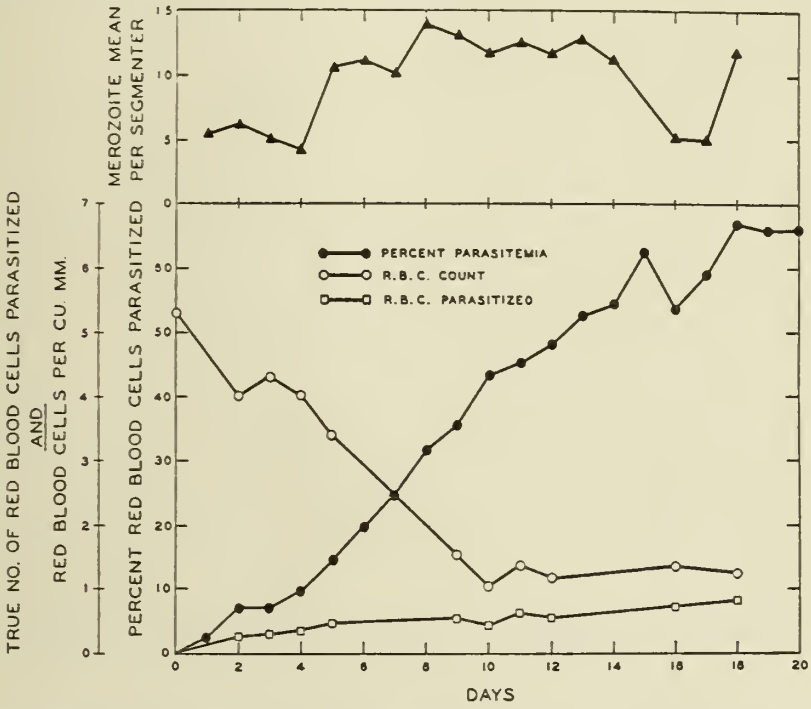

FIG. 1. Average true number of red blood cells parasitized, average red blood cells per cu. mm., average per cent red blood cells parasitized, and average merozoite mean per segmenter in *fatal* blood-induced *Plasmodium berghei* infections in white rats.

Daily counts of the number of merozoites per segmenter in the lethal infections showed that during the early course of the infection the merozoite mean was low. The range was from 5.50 ± 1.58 on the first patent day to 4.14 ± 0.35 on the fourth. This was followed by a gradual increase to a peak on the eighth day (14.1 ± 0.21); this average was more or less maintained for the succeeding six days. Thereafter, a steady decrease occurred until the sixteenth day when an average of 5.17 ± 0.66 nearly approached the early counts and was followed by a sharp increase to 12.2 ± 1.29 on the eighteenth day.

The merozoite mean in those infections which survived the acute parasitemia ranged from 3 to 8 on the second and third patent days, respectively, after which

there was a sharp rise to 16 on the fifth day. During the next 11 days there was a more or less gradual drop in the merozoite mean to 4.6 ± 0.45. A gradual progression to 9.57 ± 1.0 on the nineteenth day was subsequently observed.

From a normal value of 5.32 ± 0.15 million red blood cells per cu. mm. a marked decrease occurred to 1.06 ± 0.05 million per cu. mm. on the tenth day in the lethal infection group after which there was little change in the count until death. In the recovered animals the red count fell to as low as 0.78 on the twelfth day. This was

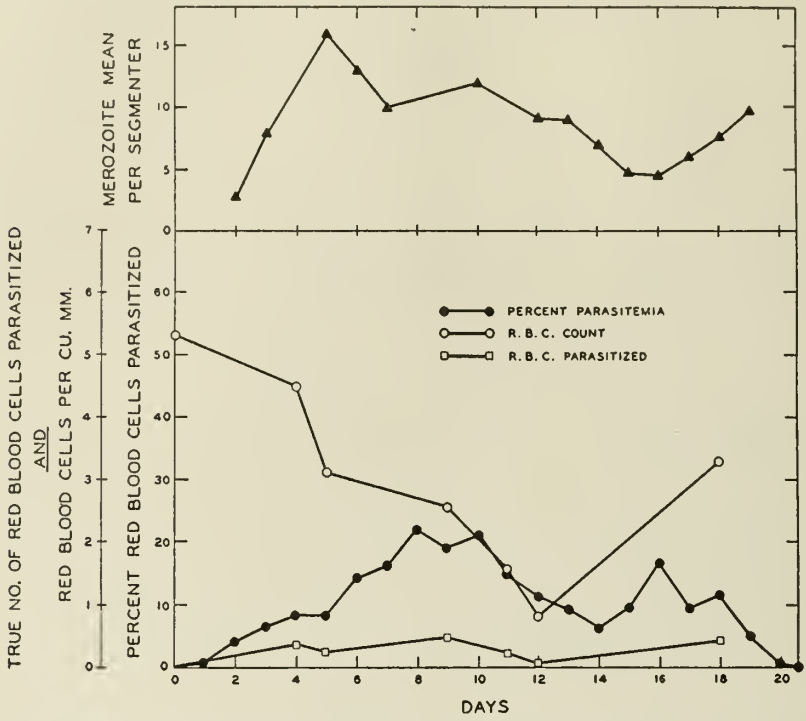

FIG. 2. Average true number of red blood cells parasitized, average red blood cells per cu. mm., average per cent red blood cells parasitized, and average merozoite mean per segmenter in *recovered* blood-induced *Plasmodium berghei* infections in white rats.

followed by a gradual increase to 3.28 ± 0.96 million per cu. mm. on the eighteenth day.

As the infection progressed the animals showed a yellowish discoloration of the skin and the normal pink color of the eyes became a yellowish white. There was considerable loss of weight and of activity, most marked near the terminal stage of the disease. Shortly before death labored breathing and somnolency occurred. In those animals which recovered, these signs were less intense and disappeared altogether following the acute attack.

DISCUSSION

In contrast to the *Plasmodium berghei* infection in the white mouse which is lethal for all infected animals (Vincke and Van den Bulcke, 1949b; Thurston, 1950; Mercado

and Coatney, 1951), the infection in the white rat is marked by two types of infection, lethal and recovered. Vincke and Van den Bulcke (1949) have shown that following intraperitoneal inoculation of blood containing the KBG 173 strain of *Plasmodium berghei* into white rats the first parasites appeared in the blood after two or three days, thereafter the parasitemia increased progressively to a maximum which was followed by either death or recovery of the animals. Mortality and recovery rates of 50 per cent were obtained, death or recovery occurred in most cases between the tenth and fourteenth day after inoculation. In the present investigation a mortality rate of 88.9 per cent and a recovery rate of 11.1 per cent were obtained; otherwise the results are in close agreement with those reported above. The parasitemia curve obtained for the lethal infections is also similar to that obtained for the infection in white mice (Mercado and Coatney, 1951), i.e. a progressive increase to a peak after which little change was observed until death. In contrast to the mouse infection, which showed a progressive increase to a peak of 34 per cent on the fifth patent day, the white rat infections exhibited a more gradual progression of the parasitemia extending to fifteen days and reaching a peak of 63 per cent.

The changes observed in the parasitemia and in the average merozoite mean per segmenter were similar to those discussed in the studies on the white mouse. After the fifteenth patent day the expected progression in the parasitemia as determined by the counts did not occur, the number of segmenters became progressively less, and the organization of the merozoites less distinct as the terminal stage of the disease approached. As in the white mouse the occurrence of this phenomenon in the white rat may have been due to an increase in the fragility of the parasitized red cells with a subsequent modification of the counts. As the infection progressed the number of disrupted cells increased probably as a result of the smearing process, although some of this disruption may have occurred *in vivo* as suggested by Shen *et al.*, (1946).

The lack of correlation between the degree of anemia observed and the parasitemia produced in the white rat after the tenth patent day is also comparable to that which occurred in the white mouse after the sixth patent day and may also be attributed to the increased fragility of the infected red cells.

Galliard and Lapierre (1951) related differences in mortality, intensity of the parasitemia, and degree of anemia to the age and weight of the animals. In a study of twelve rats, 50 per cent of the young, 50-gram animals died of the infection, while the older, 250-gram animals exhibited only a slight parasitemia and all survived. In the young rats a drop in the red blood cell count occurred from a normal of 9 million per cu. mm. to 3 million per cu. mm.; the older animals exhibited a less intense anemia with a drop to 7 million per cu. mm. In our studies a more marked decrease to approximately one million per cu. mm. occurred from a normal value of 5.32 ± 0.15 million per cu. mm. Raffaele and Baldi (1950) also noted the influence of age on the course of the *berghei* infection in white rats; very young rats died between the tenth and twenty-third day after inoculation, but in animals 30 to 60 days old the mortality rate was only 25 per cent. Corradetti (1950) attributed recovery from a *berghei* infection to the formation of an acquired immunity largely maintained by the spleen, but which could be taken over after splenectomy by the reticulo-endo-

thelial system of other organs. Baldi (1950) related the influence of immunity to the presence or absence of a sufficient number of reticulocytes in the blood stream to which the *berghei* parasites had a particular affinity; he postulated that the 100 per cent mortality in white mice was due to their ability to produce large numbers of reticulocytes with the result that the animals were overwhelmed before the immune reaction could become operative. Young rats responded in much the same manner, due to their large number of available reticulocytes, and died of the infection. In older animals the number of reticulocytes was appreciably lessened, thereby limiting the multiplication of the parasite, the immune response became operative and the animals recovered. The nature of the mechanism which permits certain of the animals to survive, in our study only 11.1 per cent, can be determined only by further study.

SUMMARY

1. Two types of infection, lethal and recovered, resulted when *Plasmodium berghei*-infected blood was inoculated into white rats, *Rattus rattus*. Lethal infections were characterized by a prepatent period of 2 to 5 days, average 3.33 ± 0.15 (S.E. of mean) days; a patent period of 8 to 21 days, average 12.2 ± 0.53 days; a peak parasitemia at 10 to 15 days, average of 11.2 ± 0.49 days. Death in most cases occurred within 24 hours after the peak. Recovered infections were characterized by a prepatent period of 2 to 5 days, average 3.2 ± 0.5 days; a patent period of 16 to 22 days, average 18.6 ± 1.18; a peak parasitemia at 7 to 16 days, average of 11.0 ± 1.64.

2. In the lethal infections the average number of merozoites per segmenter ranged from 5.50 ± 1.58 on the first patent day to 14.1 ± 0.21 on the eighth. The lowest count, 5.17 ± 0.66, occurred on the sixteenth day, after which it rose to 12.2 ± 1.29 on the eighteenth.

3. In the recovered infections the mean number of merozoites per segmenter ranged from 3 to 8 on the second and third patent days, respectively, to 16 on the fifth day after which there was a gradual drop to 4.6 ± 0.45 on the sixteenth day, and a rise to 9.57 ± 1.0 on the nineteenth day.

4. The red cell count in normal uninfected rats was 5.32 ± 0.15 millions per cu. mm. As a result of the infection this number dropped to 1.06 ± 0.09 in the lethal infections and in the recovered infections to 0.78 million per cu. mm. In the latter animals the red cell count increased to 3.28 ± 0.96 on the eighteenth day.

5. As the infection progressed the animals showed a yellow discoloration of the skin; the eyes became yellowish white; there was loss of weight and of activity. Near the terminal stage of the disease these signs became intensified and were accompanied by labored breathing and somnolency shortly before death occurred. Animals which survived the acute attack soon returned to normal.

REFERENCES

Baldi, A. 1950. Sul quadro anemico nell'infezione da *"P. berghei"* (Vincke e Lips). Riv. di Malariol. **29:** 349–356.

Corradetti, A. 1950. Particolari fenomeni immunitari nell'infezione da *Plasmodium berghei*. Riv. di Parassit., **11:** 201–209.

Corradetti, A. and Verolini, F. 1951. Relazioni tra *Plasmodium berghei* e cellule della serie rossa durante l'attacco primario nel ratto albino. Riv. di Parassit., **12:** 69–84.

GALLIARD, H. AND LAPIERRE, J. 1951. Infection a *Plasmodium berghei* chez le rat blanc. Bull. Soc. Path. Exot., **44**: 185–194.

MERCADO, T. I. AND COATNEY, G. R. 1951. The course of the blood-induced *Plasmodium berghei* infection in white mice. J. Parasit., (in press).

RAFFAELE, G. AND BALDI, A. 1950. Sulla morfologia e sulla transmissione di "*P. berghei*" (Vincke e Lips). Riv. di Malariol., **29**: 341–347.

RODHAIN, J. 1949. Le comportement du cotton rat vis-à-vis du *Plasmodium berghei* (Vincke et Lips). Ann. Soc. Belge de Méd. Trop., **29**: 483–490.

SHEN, S. C., FLEMING, E. M., AND CASTLE, W. B. 1946. Osmotic and mechanical fragilities of erythrocytes of monkeys infected with *P. knowlesi* malaria. Proc. Soc. Exp. Biol. and Med., **63**: 419–422.

THURSTON, J. P. 1950. The action of antimalarial drugs in mice infected with *Plasmodium berghei*. Brit. J. Pharm. and Chemo., **5**: 409–416.

VINCKE, I. H. AND VAN DEN BULCKE, M. A. 1949. Réaction des *Rattus rattus* vis-à-vis du *Plasmodium berghei*, Vincke et Lips. Ann. Soc. Belge de Méd. Trop., **29**: 537–544.

VINCKE, I. H. AND VAN DEN BULCKE, M. A. 1949a. Réaction des *Thamnomys surdaster surdaster* vis-à-vis du *Plasmodium berghei*, Vincke et Lips. Ann. Soc. Belge de Méd. Trop., **29**: 545–548.

VINCKE, I. H. AND VAN DEN BULCKE, M. A. 1949b. Observations sur l'entretien de la souche K173 du *Plasmodium berghei*, Vincke et Lips. Ann. Soc. Belge de Méd. Trop., **29**: 525–536.

RESUMEN

1. Observáronse dos tipos de infección, las mortales y las recuperadas, en ratas blancas, *Rattus rattus*, después de inyectárseles con *Plasmodium berghei*. La infección letal se caracterizó por un período prepatente de 2 a 5 días, promedio 3.33 ± 0.15 (E.S. del promedio) días; un período de infección patente de 8 a 21 días, promedio 12.2 ± 0.53 días; una parasitemia máxima en 10 a 15 días, promedio 11.2 ± 0.49 días. En la mayoría de los casos la muerte ocurrió dentro de 24 horas después que se registró la parasitemia máxima. Las infecciones recuperadas se caracterizaron por un período prepatente de 2 a 5 días, promedio 3.2 ± 0.5 días; un período de infección patente de 16 a 22 días, promedio 18.6 ± 1.18; una parasitemia máxima en 7 a 16 días, promedio 11.0 ± 1.64.

2. En la infecciones mortales el promedio de merozoítos por parásito segmentado se extendió de 5.50 ± 1.58 en el primer día de infección patente a 14.1 ± 0.21 en el octavo día. El contaje menor, 5.17 ± 0.66, ocurrió en el decimosexto día y aumentó a 12.2 ± 1.29 en el decimoctavo día.

3. En la infecciones que recuperaron el promedio de merozoítos por parásito segmentado se extendió de 3 a 8 en el segundo y tercer día de infección patente respectivamente a 16 en el qunto día después del cual ocurrió un descenso gradual a 4.6 ± 0.45 en el decimosexto día y un aumento a 9.57 ± 1.0 en el decimonoveno día.

4. Se observó un contaje de glóbulos rojos de 5.32 ± 0.15 millones en ratas normales no infectadas. Como resultado de la infección esta cantidad se redujo a 1.06 ± 0.09 en las infecciones mortales y en las recuperadas a 0.78. En éstas la cantidad de globulos rojos aumentó a 3.28 ± 0.96 en el decimoctavo día.

5. Durante el progreso de la enfermedad la piel de los animales se volvió amarillenta, los ojos de un color blanco amarillento, se redujo su peso normal y su actividad. Estos signos se volvieron más intensos al acercarse la muerte y fueron acompañados por respiración forzada y somnolencia poco antes de morir. Aquellos animales que sobrevivieron el ataque águdo de la enfermedad pronto recobraron su estado normal.

Professor Alberto Missiroli

1883–1951

Professor Alberto Missiroli was born in the town of Cervia, province of Ravenna, on the 27th of July, 1883 and died in Rome on the 18th of July, 1951, after a notable career in parasitology, especially in the study and control of malaria.

Having received his doctorate in 1908 at Bologna, where his work on thyroid function, at the Institute of Pathologic Anatomy won high praise, Missiroli became an Assistant in Legal Medicine. Soon, he developed an interest in public health bacteriology, volunteering as an assistant at the Institute of Hygiene, University of Siena, in 1910. During the same year he helped the Public Health Department of Puglie to suppress a cholera epidemic. In 1911 he went to the bacteriology laboratory in Sassari, Sardinia, and in 1913 to Tripoli, in both areas studying undulant fever and publishing significant papers. He joined the public health laboratories in Rome as an Assistant in 1914 and in 1916 helped to set up routine courses in preventive medicine.

In 1918 Missiroli founded The School of Malaria at Nettuno, on the coast at the western edge of the Pontine Marshes, and so he began his span of 33 years as a malariologist. His studies in Nettuno led to a series of publications relating to *Anopheles* and to malaria epidemiology and control. This School was officially closed in 1935.

In 1924 Missiroli became associated with L. W. Hackett and together they carried out their fundamental malaria studies under the joint auspices of the Italian authorities and the International Health Division of The Rockefeller Foundation. The world-renowned "Stazione Sperimentale per la Lotta Antimalarica" was organized in 1926. To its laboratories and field areas, during its 11 years of activity, came observers and students from every continent, representing national health departments and such institutions as the London and Hamburg Schools of Tropical Medicine, the Paris Institute of Parasitology, and the Koch Institute in Berlin. In 1929 the undersigned spent six very profitable weeks in Italy with Hackett and Missiroli, an experience still vivid in my memory.

From 1924 to 1939 Missiroli and Hackett and their colleagues were busy elucidating the epidemiology of European malaria, unlocking secrets of the *maculipennis* complex which had puzzled entomologists for so long, and demonstrating the benefit and practicality of malaria control by the anti-anopheline measures of Paris green larviciding and drainage. Rarely has an association been more fruitful in scientific discoveries and practical results than that between Missiroli and Hackett.

In 1934 the new Istituto Superiore di Sanità Pubblica was opened in Rome and Missiroli became director of the malariology division which was enlarged to the present division of parasitology in 1948. Gathering around him Mosna, La Face, Corradetti, Gramiccia, Bettini, Saccà, and others he built up an unusually active teaching and research unit which has greatly enlarged our knowledge of the epidemiology and control of insect-borne disease. This group, for example, was first to detect the now well-known phenomenon of mosquito and fly resistance to DDT.

During World War II Prof. Missiroli, in Rome, did what he could to maintain some semblance of his organization and to hold back the upsurge of malaria among the people of the Pontine Marshes and the Rome Campagna. The forces of war had disrupted all public services and the retreating enemy, in spite of Missiroli's strong pleas to high-ranking German medical officers, had sabotaged anti-malaria engineering works, drainage, and pumping systems from the Fondi Marshes to Maccarese. Missiroli used atabrine skillfully as a suppressive and therapeutic drug so that in the areas under his control he was able to prevent serious malaria mortality.

When the author of this biographical note arrived in Rome with the Allied Forces, early in June, 1944, the first individual he sought out was Missiroli. The latter responded enthusiastically and rendered great assistance to the Allied Control Commission in the re-establishment of malaria control in Italy. After responsibilities for aiding civilian public health were transferred from the ACC to UNRRA, Missiroli continued to give advice and active help.

In 1946 Missiroli published a five-year plan for the complete eradication of malaria from Italy. Many considered his project to be fantastic and few believed that it could be accomplished. But Missiroli had been studying the effect of DDT, as demonstrated by the ACC for two years, and this experience together with his thorough knowledge of the epidemiology of malaria in his country and his long service in the training of Italian malaria workers, indicated to him that it could be done. Fortunately, on the

basis of his reputation, he persuaded the authorities to implement his plan, with the result that at the time of his death, during the fifth season of his nation-wide scheme, malaria in Italy had become a rare disease. This was Missiroli's crowning glory. He displayed not only sound knowledge but also clear vision and real courage. The elimination of malaria from Italy will have a place among the greatest achievements in the history of malaria.

In 1947, Missiroli set up in Latina a field station for the study of insecticides. Here sprays and sprayers have had thorough testing and students have been trained. This station has proved to be a very useful supplement to the Rome laboratories and is still in active use.

Missiroli represented his country at the 4th International Congresses of Tropical Medicine and Malaria in 1948 in Washington. He was a member of the Malaria Panel of the World Health Organization and a co-opted member of the WHO Expert Malaria Committee at its Third Session which met in Geneva in 1949. It will be recalled that Missiroli had also participated actively in the work of the Malaria Commission of the Health Organization of the old League of Nations, before World War II.

Missiroli was a member of many scientific societies at home and abroad and was the author and co-author of some 150 scientific papers. At the time of his death he was Editor of the Rivista di Parassitologia which he founded in 1937.

Looking back over the years, it seems apparent that Missiroli had a large part in determining the precise vectors among the confused *maculipennis* complex, in bringing about European acceptance of the principle of species sanitation, in training a large corps of malaria survey and control personnel, in stimulating malaria research in laboratory and field, and finally in originating plans, developing organization, and providing leadership for the eradication of malaria from Italy by residual DDT spraying. Few scientists have ever progressed so logically, so persistently, and so successfully toward a major goal. Italy and the world have sustained a great loss.—*Paul F. Russell.*

BOOK REVIEW

Malaria and Its Control in Bombay State. D. K. Viswanathan. 263 pp. with 22 illustrations; 5 pp. of advertisements. D. K. Viswanathan, Connaught House, Poona, Bombay State, India. 1950.

This monograph is a history of the Malaria Control Organization in the Bombay State from its inception until 1950. It is written in an interesting and sometimes provocative manner. The development of this type of Public Health program will be of interest to all who are working in the tropics. The striking change in the attitude of the public as the control program developed is noteworthy. Before spraying with DDT, the natives only passively accepted government representatives. After realizing the obvious benefits of residual spraying with DDT, the natives were enthusiastic in welcoming the spraying teams and gave cooperation which made for a much more thorough job.

In the first section the organization of the Bombay State Malaria Control work is presented. The second section takes up the pre-DDT period presenting data on the epidemiology of malaria in certain districts, the behavior of *Anopheles fluviatilis*, experiments in the chemotherapy of malaria, and experimental control measures before the advent of DDT. It was pointed out that *A. fluviatilis* apparently varies its habits according to the locale so that in one district it was not necessary to spray cattle sheds but spraying was necessary in another district. Infant mortality rates are considered important in assaying malaria incidence.

The response of parasites to paludrine is presented. This drug apparently clears the asexual parasites readily from the blood stream but does not seem to adversely influence the number of gametocytes, especially those of *Plasmodium falciparum*. Drup suppression is not considered to be a satisfactory control measure. It is suggested that the best results are obtained by using DDT for the control of mosquitoes and Paludrine (chlorguanide) for the treatment of clinical cases.

The third section is entitled "The DDT Period" and follows the extension of the residual sprays of DDT from the pilot experiments until its use in many of the districts. After the institution of DDT residual spray, not only was there a drop in the spleen and parasite rates for malaria but there was a significant decrease in malaria death rates and a significant increase in the birth rate. Furthermore, DDT seemed to reduce plague. It was effective against both *A. fluviatilis* and *A. culicifacies* even though their habits differed considerably.

Urban malaria control is considered in the fourth section. In urban areas above 40,000 population, anti-larval control may be more economical than residual spray. However, if other insect-borne diseases are important, this might not hold true.

Malaria surveys are presented in section five, beginning with a general consideration of the epidemiology of malaria in Bombay State and then presenting surveys of several of the districts The results of an anopheline survey of the Bombay State are presented. Twenty-seven anophelines have been met with and their distribution and relative abundance are shown in a table.

The final section is on future plans and aspirations which includes a lot of practical philosophy about malaria control. Cost figures for malaria control are given. Throughout the monograph runs a thread of reasoning to show that malaria control pays. Considering the earning capacity of those who might be prevented from having malaria, should the control scheme be extended to the whole state of Bombay, it is estimated that annual savings could amount to 750,000,000 rupees. The investment in malaria control pays dividends fiftyfold!

The printing and editing of this monograph are poor. However, the material is interesting and the facts presented are convincing. It is recommended to all of those who are interested in malaria. —*Martin D. Young.*